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THEME

Antibacterial effects of raw extracts, aloïn, and phynolic compounds of Aloe Vera latex on different strains

(*Escherichia coli, Staphylococus aureus* and *Pseudomonas aeruginosa)*

THE JURY

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Dedications

I dedicate this modest work as a proof of love:

First I want to thank Allah foe evrything gave me in my life

To the most dearest being of my life, my mother

To the one who made me a man, my father ***HADJ ABBAS***

Allah keep them safe for me.

To my dear older brother ***Khaled*** *and sisters and their children*

Want thank all my family members for supporting me uncels ,aunts and their children

And to all the students of the master 2 LMD/BVP class of the year 2020-2021

To you dear reader

Abbreviation:

PP: Polyphynole.

ENT: Ears, Nose and thraot

FDA: Food and Drug administration.

% : Percent.

°C : Celsius.

DPPH : 2 ,2-diphenyl-1-pienythydrazyl.

UV : Ultra violet

DMSO: Dimethyle sulfocide.

DM: Dulition mother.

MH : Mueller-Hinton.

P aerogenosa : Pseudomenas Aerogenosa.

S aureus : Staphylococcus Aureus.

E coli: Escherichia coli.

Ph: Potential of hydrogen

USA: united States of America

Cm: Centimeter

MPS: Mucopolysaccharides.

NADPH: Nicotinamide adenine dinucleotide phosphate

Ampi:Ampicilin

PCL: Phynolic compounent of latex

Abstract:

The pharmacological potential of plants, especially Aloe Vera, a medicinal plant native to South and East Africa, traditionally and widely used for thousands of years, with numerous therapeutic properties, it has been considered a miracle plant. There are three parts in the leaf: the juice containing many anthraquinones, with laxative properties, the latex, and the gel which is healing, moisturizing, anti-inflammatory, anti-infectious, anti-allergic, anti-diabetic and even antiviral. These numerous components have been used in synergy for a long time, their effects confirmed by numerous scientific studies. In this context and taking into account the medicinal knowledge of the natives, we have outlined our objective which is to test the synergistic antibacterial effect of the polyphenols contained in the whole leaf against (*Pseudomonas areuginosa, Staphylococcus aureus* and *Escherichia coli*), and compare it with the same effect of the active molecules extracted from each part of the leaf separately. In this perspective, we proceeded to the extraction by maceration of each part separately and the whole leaf; which showed a yield of the PPs extracted with annonk method.

Their antibacterial activities of the different extracts were estimated by inhibition on MH agar, and plotted diameters of inhibition showed significant sensitivity anti-bacterial effects with $11,5\pm2,355^{b}$ of aloin diluted 50% against the E coli strains, and for the *S aureus* showed sensitive anti-bacterial effects with diameter $13,083\pm1,165^{b}$ by the aloin pure in other hand *P aeroginosa* showed a resistant to all the PPs with diameter of initiation less then $7,5\pm4,964^{a}$ in thynolic compounds of latest as the highest value .This reveals an antibacterial effect by bactericidal or inhibitory action very interesting.

Aloe Vera present excellent alternatives to synthetic antibiotics to fight against bacterial infestations caused by *E coli* and *S aureus*, allowing us to face the problems of antibiotic resistance and the dramatic side effects of the excessive consumption of synthetic antibiotics.

It is just important to underline that the use of the whole leaf is not without risk as toxicity problems have also been found.

Keyword: Aloe Vera, latex, aloin, anthraquinons, gel and polyphenol.

ملخص:

الإمكانات الدوائية للنباتات ، وخاصة الألوة فيرا ، وهو نبات طبي موطنه جنوب وشرق إفريقيا ، يستخدم تقليديًا وعلى نطاق واسع لآلاف السنين ، مع العديد من الخصائص العلاجية ، وقد تم اعتباره نباتًا معجزة. هناك ثلاثة أجزاء في الورقة: العصير الذي يحتوي على العديد من الأنثراكينون ، مع خصائص ملين ، واللاتكس ، والهلام الذي يشفي ، ويرطب ، ومضاد للالتهابات ، ومضاد للعدوى ، ومضاد للحساسية ، ومضاد للسكري وحتى مضاد للفيروسات. تم استخدام هذه المكونات العديدة في التآزر لفترة طويلة ، وأكدت العديد من الدراسات العلمية آثارها. في هذا السياق ومع الأخذ في الاعتبار المعرفة الطبية للسكان الأصليين ، حددنا هدفنا وهو اختبار التأثير المضاد للبكتيريا المتآزر للبوليفينول الموجود في الورقة بأكملها ضد *Pseudomonas aeruginosa*) و *Staphylococcus aureus*و (*Escherichia coli* ومقارنته بنفس تأثير الجزيئات النشطة المستخرجة من كل جزء من الورقة على حدة. من هذا المنظور ، شرعنا في الاستخراج عن طريق النقع لكل جزء على حدة والورقة بأكملها ؛ التي أظهرت إنتاجية PPs المستخرجة بطريقة أنونك.

تم تقدير نشاطهم المضاد للبكتيريا للمستخلصات المختلفة عن طريق تثبيط أجار MH ، وأظهرت أقطار التثبيط حساسية كبيرة مضادة للبكتيريا مع 11.5 ± 2.355 bمن الألوين المخفف بنسبة 50 ٪ ضد سلالات *E coli* ، وبالنسبة لـ *S aureus* أظهرت تأثيرات حساسة مضادة للبكتيريا بقطر 13،083 ± 1،165 bبواسطة aloin pure في جهة أخرى ، أظهر *P aeroginosa* مقاومة لجميع PPs بقطر بدء أقل من 7،5 ± 4،964 aفي مركبات thynolic الأحدث كأعلى قيمة. هذا يكشف عن تأثير مضاد للجراثيم من خلال عمل مبيد للجراثيم أو مثبط مثير للاهتمام.

تقدم الألوة فيرا بدائل ممتازة للمضادات الحيوية الاصطناعية لمحاربة العدوى البكتيرية التي تسببها بكتيريا *E coli* و *S aureus* ، مما يسمح لنا بمواجهة مشاكل مقاومة المضادات الحيوية والآثار الجانبية الدراماتيكية للاستهلاك المفرط للمضادات الحيوية الاصطناعية.

كلمات مفتاحية :اليوفيرا، الاتكس، الالوين، انتراكينون، الجيل.

من المهم فقط التأكيد على أن استخدام الورقة بأكملها لا يخلو من المخاطر حيث تم العثور على مشاكل سمية أيضًا

Résumé:

Le potentiel pharmacologique des plantes notamment l'Aloès Vera, plante médicinale originaire de l'Afrique du Sud et de l'Est, traditionnellement et largement utilisée depuis des millénaires, aux nombreuses propriétés thérapeutiques, elle a été considérée comme plante miracle. On distingue trois parties dans la feuille : le suc contenant de nombreuses anthraquinones, aux propriétés laxatives, le latex, et le gel qui est cicatrisant, hydratant, anti-inflammatoire, anti-infectieux, antiallergique, antidiabétique et même antiviral. Ces nombreux composants ont été exploités en synergie depuis longtemps, leurs effets confirmés par de nombreuses études scientifiques. Dans ce contexte et en tenant compte du savoir médicinal des autochtones, nous avons tracé notre objectif qui est de tester l'effet antibactérien synergiques des polyphénols contenus dans la feuille entière vis-à-vis (*Pseudomonas areuginosa*, *Staphylococcus aureus et Escherichia coli)*, et le comparer avec le même effet des molécules actives extraites de chaque parti de la feuille séparément. Dans cette optique, nous avons procédé à l'extraction par macération de chaque partie séparément et la feuille entière ; qui a montré un rendement des PPs extraits avec la méthode annonk. Leurs activités antibactériennes des différents extraits ont été estimée par inhibition sur gélose MH, et ont tracé des diamètres d'inhibition a montré des effets antibactérienne de sensibilité significative avec 11,5±2,355b d'aloïne diluée à 50 % contre les souches *E coli*, et pour le *S aureus* a montré des effets antibactériens sensibles avec un diamètre de 13 083±1,165b par l'aloïne pure d'autre part *P aeroginosa* a montré une résistance à tous les PP avec un diamètre d'initiation inférieur à 7,5±4 964a dans les composés phénolique de la dernière valeur la plus élevée Ceci révèle un effet antibactérien par action bactéricide ou inhibitrice très intéressent.

L'Aloès- Vera présentent d'excellents alternatifs aux antibiotiques de synthèses pour lutter contre les infestations bactériennes causées par *E. coli et S. aureus*, nous permettant ainsi de faire face aux problèmes d'anti-biorésistance et des effets secondaires dramatiques de la consommation excessive des antibiotiques de synthèse.

Il est juste important de souligner que l'utilisation de la feuille entière n'est pas sans risque car des problèmes de toxicité ont également été retrouvés.

Mots clé : Aloe Vera, latex, aloin, anthraquinons, gel and polyphénol

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GENERAL INTRODUCTION

General introduction

Traditional medicine has been practiced for many centuries by a substantial proportion of the population for many centuries.

Most plant species have medicinal value and have been characterized since ancient times, causing no toxic effects on the human body (Mothana, et al., 2005).

Aloe Vera is one of the oldest plants mentioned because of its medicinal properties and health benefits. Ancient physicians considered this plant to be a blessing to mankind. Often called a "miracle plant" or "nature's healer", Aloe Vera is a plant with many surprises. The botanical name of Aloe Vera is Aloe Barbadensis miller. It belongs to the family Asphodelaceae (Liliaceac).The name 'Aloe' comes from the Arabic word 'alloeh' or the Hebrew word 'halal', meaning bitter and shiny substance; 'Vera' in Latin means 'real'. Because of its cactus-like feel, Aloe is often mistakenly called a "Desert Cactus". There are over 400 species of Aloe cultivated in the world, but it is the Aloe barbadensis (Aloe Vera or "True Aloe") that has been most useful to mankind because of its medicinal properties (Mehta, 2017).

Aloe Vera contains different nutritional contents such as vitamins, minerals, enzymes, sugars, phenol compounds, lignin enzymes, sugars, phenol compounds, lignin, saponin, sterol and amino acids.

It is widely used in health care and cosmetic products (Natacha, 2013) (Surjushe, et al., 2008). Aloe Vera has properties that have many medicinal uses. It has been observed through research that taking Aloe Vera in food or drinks has reduced the blood glucose level which has been helpful in controlling diabetes. Most people with diabetes consumed aloe vera mixed with yogurt or in the form of herbal tea. It has also been used in anti-aging. It can be applied to get relief from sunburns or other types of burns as it reduces pain, inflammation, relieves the burning sensation and heals the wound very quickly (Sampath Kumar, et al., 2010)

Since their discovery at the beginning of the 20th century, antibiotics have allowed great advances in therapy and contributed to the development of modern medicine. The introduction and clinical use of the first classes of antibiotics significantly reduced mortality from previously incurable diseases. The effectiveness of antibiotic therapy in controlling and limiting the spread of pathogens has thus given rise to the hope of being able to eradicate all infectious diseases. Unfortunately, the emergence of antibiotic-resistant bacteria put an end to this wave of optimism.

the antibacterial effect of the pholyphynol of Aloe Vera with antibacterial activity and to search, in the reservoir of chemical molecules that they constitute, for compounds capable of inhibiting the growth of pathogenic bacteria, in order to use them as alternatives to antibiotics of widely used as a treatment for different microbial infections

My objective is to present study aims particularly to proceed to the extraction of polyphynol from Aloe Vera, especially the aloin and phenolic compounds of the latex by the use of Annok method (Arnnok, et al., 2012). In order to study the antibacterial activity of these different PPs pure and at different dilutions on gram negative pathogens, *Pseudomonas aeruginosa*, *Escherichia coli*, and gram positive bacteria, *Staphylococcus aureus.*

This study is subdivided into five chapters:

- The first chapter consists of a bibliographical study.
- The second chapter consists of the polyphynols.
- The third chapter about the bacterial strains.
- The fourth chapter consist material and methods.
- The fifth chapter is devoted to the presentation of the results obtained and their discussions.

CHAPTER I

GENERALITIES ABOUT ALOE VERA

1.1 History:

Aloe Vera is a favorite plant of many nations of the world. It has been found and described in the writings of many different cultures and as far back as Greek, Egyptian and Roman times... References have also been found in the writings of early Indian and Chinese cultures. It has been one of the most widely used and wanted plants throughout history (Mehta, 2017).

The Aloe Vera plant has a history dating back to biblical times and, belonging to the lily family, is a cactus-like perennial plant (Surjushe, et al., 2008).

Throughout the ages, Aloe Vera has been revered by many civilizations and cultures. So much so that it has been called a holy plant and symbolized "beauty, health and wellness". At present, no one can really say how long ago it was first used as a medicinal plant, but there is evidence that it has been used for more than 5,000 years in regions of the world as far apart as southern Europe, Asia, northern Africa, America and the Middle East, Africa, America and the Far East (Benzie, 2011).

As a symbol of immortality, the ancient Egyptians were convinced that Aloe Vera had the power to facilitate the passage of the deceased into the other world. They placed Aloe Vera leaves in the tombs of their deceased pharaohs so that their journey would be under the best of omens (Haller Jr, 1990).

The first historical mention of the use of this plant as a cosmetic is probably that of the Egyptian queen "Cleopatra", who secretly used Aloe Vera gel in her bath as a beauty treatment and youth enhancer. (Haller Jr, 1990)

The Arabs knew since the highest antiquity the virtues of the Aloe which they call "Lily of the desert". 600 years before Christ, the Arab civilization was one of the first to describe two different juices and develop a process for separating the gel from the sap: using their bare feet, the Arabs crushed the Aloe Vera leaves and placed the resulting paste in and placed the resulting paste in goatskin bags. These bags were then placed in the sun so that the contents could dry out completely and then be ground into powder. These resin extracts, which were used mainly as a laxative, but also for many other internal and external uses, contributed greatly to the external uses, have largely contributed to the diffusion of Aloe in many countries of the Middle East and Asia (Natacha, 2013).

For the Greeks, aloe Vera symbolized beauty, patience, fortune and health discords, an ancient Greek physician and botanist, described the properties of the plant in his treatise "De Material Media". It was used for healing; treatment of boils, irritations of the ENT sphere, dry and irritated skin, genital ulcers, and for stopping bleeding (Natacha, 2013).

The Hindu's call Aloe Vera "the silent healer", the ayurveda medicine of India holds Aloe Vera in high esteem, as a major plant in its pharmacopoeia. Considered a sacred plant, some of its species were strictly protected, its gel was used as a healer in surgery (application of the raw gel on the incision) to promote rapid healing, better and natural (Benzie, 2011).

1.2. The different species of Aloe:

Nowadays, there are more than 500 different species of Aloe, with very different sizes and appearances. Some species of Aloe are very popular houseplants because they are versatile and able to thrive indoors without much care. This is beneficial because this is beneficial because living plants like Aloe can improve air quality and help promote a healthier indoor environment. Among the different species of Aloe (Zapataa P.J, 2013); there are:

1.2.1. Aloe Ferox:

Aloe Ferox is also known as "aloe de capa", "aloe rojo" or "aloe de Grifo". It can grow to It can grow up to 10 feet (3 meters) in height, its red flowers grow 2 to 4 feet above its (2 to 4 feet) above its leaves (2 to 4 feet). Extracts of this plant have natural laxative properties and studies show that it is studies show that it is effective against occasional constipation. Researchers have discovered that the seed oil contains high levels of linoleic, stearic and oleic fatty acids and oleic fatty acids used in many cosmetics and offers a natural way to nourish and rejuvenate the skin (figure1) (Zapataa P.J, 2013).

Figure 1: Aloe ferox (Zapataa P.J, 2013).

1.2.2. Aloe Arborescens:

Often referred to as the "aloe candelabra", the aloe tree can grow up to 3 meters high and become as large as a small tree (figure 2). Bright red-orange cylindrical flowers raise high from the plant's leaves to give it a distinctive appearance.

As with many Aloe plants, researchers report numerous healing properties. Aloe arborescence helps to heal wounds in animals and acts against harmful organisms. An Italian study has also shown that when used as a nutritional therapy, it helps the immune system and has other health benefits (Zapataa P.J, 2013).

Figure 2 : Aloe Arborescens (Zapataa P.J, 2013) .

1.2.3. Aloe Aristata:

Stemless aloe, also known as "lace aloe" and "guinea fowl aloe"(figure 3), is known for its intense green color, its green color, serrated leaves and unique white spots. It is very similar to another common succulent Haworth, and is often confused with this distant cousin. Its large orange flowers attract a wide variety of birds and insects, especially bees, which promotes the health and longevity of this and other plants in its habitat. This makes it makes it an excellent, low maintenance garden plant that thrives in both warm and cool climates. Aloe Aristata also has therapeutic properties, as it is used for wound healing in Ayurveda (Traditional Indian Medicine) (Zapataa P.J, 2013).

Figure 3: Aloe Aristata (Zapataa P.J, 2013) .

1.2.4. Aloe Ciliaris:

Aloe Ciliaris, commonly referred to as "Common Climbing Aloe", is a slender, hardy plant known for its known for its incredibly fast growth. It also has tubular red flowers and soft, hairy soft, hairy teeth. Aloe Ciliaris is an Aloe that works well in a garden, as it is known to attract bees and birds which enrich and support the life of other plants around it (figure 4) (Zapataa P.J, 2013).

Figure 4: Aloe Ciliaris (Photo Alamy Stock, 2016)

1.2.5. Aloe Maculata:

The sap of Aloe Maculata, called "Aloe Vera soap"(figure 5), forms a soapy lather in water. This species of Aloe can be recognized by its long tubular flowers which vary in color from red to green with spots that resemble the letter 'H'.

A unique feature of this particular Aloe species is that its pollen production can be increased by smoking.

This makes Aloe Maculata a popular choice among gardeners who are able to use this plant's unique ability to maintain its closest environment (Zapataa P.J, 2013).

Figure 5: Aloe Maculata (Zapataa P.J, 2013)

1.2.6. Aloe Barbadensis Miller:

Also known as: Aloe Vera (Linnaeus) or Aloe Vulgaris (Lamarck), this species and called the miracle plant and we have devoted our study in this research to the latter research on this last one (figure 6) (Zapataa P.J, 2013).

Figure 6: Aloe Barbadensis Miller (vdi, 2021)

1.3. Etymology:

Aloe Vera (L.) Burm, named and described by Linnaeus, is also known as Aloe barbadensis Miller or Aloe vulgaris Lamark.

Today, the official botanical classification has retained the name Aloe barbadensis Miller, but Aloe Vera remains the common name, which we will adopt throughout the thesis (Ernst, 2005).

Today, many common names are attributed to Aloe Vera: Aloe, real Aloe, Barbados Aloe, Aloe Vera vulgaris, desert lily, doctor of heaven, doctor plant, healing plant, miracle plant, first aid plant, burn plant, remedy harmony, Plant Doctor, Green Doctor, Aloe Doctor, Pot Doctor, Silent Healer, Fountain of Youth, Elixir of Long Life, Staff of Heaven, Gift of Venus, Plant of Immortality, Plant that Heals all (ESHUN, et al., 2004).

1.4. Geographical distribution:

The name Aloe comes from the Arabic word Alloeh meaning a brilliant bitter substance. Aloe vera is a cactus-like plant that grows easily in hot, dry climates and is currently cultivated in large quantities due to the high demand. It grows mainly in the dry regions of Asia, Africa, America and Europe (Sanghi, 2015).

1.5. Botanical description:

Aloe Vera or Aloe Barbadensis Miller is a green plant of the Liliaceae family with fleshy, cactus-like leaves, native to South Africa. Also known as "The Desert Lily", this plant is easy to grow because despite the fact that it grows outdoors in warm countries, it can also be grown indoors in pots all over the world. It is in fact a succulent, tree-like perennial of about 80 to 100 cm in height with short, shallow roots (Natacha, 2013).

1.6. Taxonomy and nomenclature:

- According to the classification of conquest (1981):

The conquest classification is a system of classification of Angiosperms. It is the latest version of the major classifications. It is essentially based on morphological, anatomical and chemical criteria. Thus, plants with a high number of similarities are grouped together in the same family (Natacha, 2013).

- Classification (Baruah, 2016):

Kingdom: Plant
Division: Tracheophytes
Branche: Spermaphytes
Sub-branch: Angiosperm
Class: Monocotyledons
Order: Asparagus
Family: Liliaceae
Subfamily: Asphodelaceae
Kind: Aloe
Species: Aloe Vera Barbadensis Miler

1.7. Plant constituents:

1.7.1. The leaf:

The leaf is the most commonly used part of the Aloe Vera plant (figure 7); a bark covers the entire plant and underneath this bark is a thin vascular layer in the form of a yellow gel. Then, inside, there is a white pulp. It is therefore possible to differentiate three separate parts (ESHUN, et al., 2004):

- The bark
- latex
- The pulp

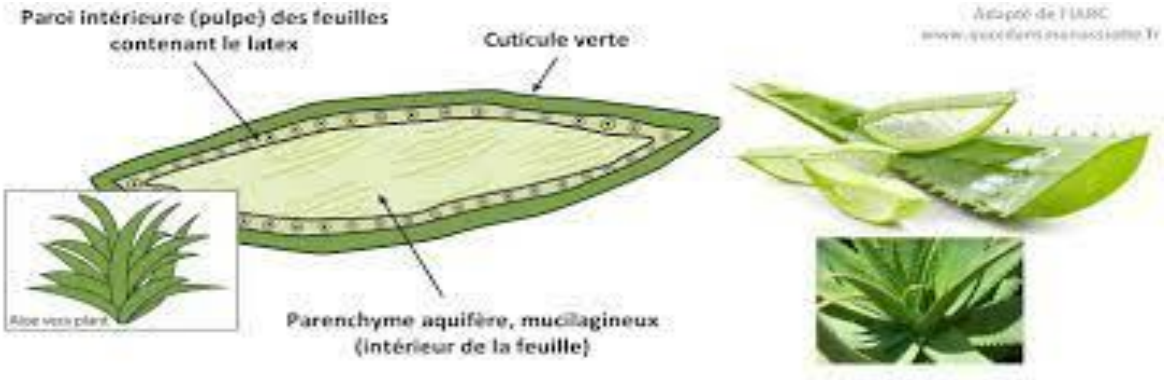


Figure 7 : cross section of aloe Vera leaf (assiette, 2018)

a) The Bark:

The bark is the outer part of the leaf, representing 20% to 30% of its weight. This part of a characteristic green color, is composed of eighteen layers of cells with chloroplasts where lipids, carbohydrates and proteins are synthesized (Guo, et al., 2016).

b) The latex:

Just below the bark is the sap of Aloe Vera, also known as latex. This bitter yellow mucilage is rich in phenolic compounds (including anthraquinones). It is the vascular system of the plant and allows, among other things, the transport of water, minerals and molecules synthesized in the roots to the pulp. Once dehydrated, this latex is used as a laxative agent regulated by the FDA. It can also be used as a bettering agent in some beverages and is considered an antibacterial, especially against Gram + bacteria (Boudreau, et al., 2004) (Of, et al., 2016).

c) The pulp:

The white, slimy part inside the leaf is made up of thin-walled parenchymatous cells containing the Aloe Vera gel. It represents 65% to 80% of the plant's weight. This clear gel serves as an energy reserve, depending on the study, it contains between 98% and 99.5% water as well as carbohydrates synthesized and stored by the plant. (Femenia, et al., 1999) (Boudreau, et al., 2004) (ESHUN, et al., 2004). The pH of Aloe Vera gel is between 4.4 and 4.7. This acidity may be due to the formation of acidic organelles such as malic acid by the plant (Boudreau, et al., 2004).

1.7.2. The flowers:

The flowers of the Aloe Vera are an erect cluster that can reach a meter in height, with numerous flowers surrounded by small yellow (sometimes orange) trumpet-shaped bracts that bloom in succession. The fleshy, orange-yellow perianth has six pieces, about 2.5 centimeters long, fused into a tube at the base. There are six stamens, slightly longer than the perianth, surrounding the three-located free ovary, which gives a locular capsule. There are six stamens, slightly longer than the perianth, surrounding the free, three-celled ovary, which produces a capsule, loculicid (the opening of a capsule by the longitudinal rupture of the midrib of the carpels), containing numerous seeds with a fleshy albumen (Perrot, et al., 1971).

1.8. Producing and planting:

Vegetative propagation is preferred to seeds for the cultivation of Aloe Vera. In Indeed, the emergence of seedlings remains poor compared to the initial growth of the shoots which is

faster. A decrease in shoot formation can be caused by water restriction. Water restriction. These can be cut off from the mother plant when they reach 15-20cm in length. long. They can be grown in a field or plot of land reserved for the propagation and cultivation of this plant during and cultivation of this plant during the first year, this is called culture nursery.

In vitro regeneration of basic leaf explants and in vitro micro propagation of meristems the world's leading producers of Aloe Vera have thousands of plants.

Aloe Vera producers in the world have thousands of hectares of plantations where the plant is cultivated and processed, from nurseries to ready-to-use products, in compliance with the most demanding production standards. Countries such as Mexico, North America and Vietnam practice extensive cultivation based on low soil productivity, without chemical inputs, drainage and watering, over large areas, and therefore characterized by low yields per hectare. In the USA, greenhouse cultivation is preferred. Other companies subcontract the cultivation of Aloe Vera to independent plantations (figure 8) (Schmelzer G.H., 2008).

Figure 8: Photo of a field of Aloe Vera plants in the Canary Islands (Jemenez, 2016)

1.8.1. Cultivation conditions:

a. The soil:

Aloe Vera grows best on dry, sandy, alkaline or neutral soils. It can grow on nutrient-poor soils but thrives on rich soils. It also has a good salt tolerance (Grindlay R., 1986).

b. The sunlight:

Sunlight, although necessary for plant growth, should not be excessive. Overexposure would result in a weak plant with low gel content.

Shade is therefore important for good development and it is therefore recommended to plant Aloe Vera between other crops such as fruit trees for example and even if it can survive at a temperature of -3°C with little damage, this technique allows to fight against the strong frosts which can be devastating (Grindlay R., 1986).

c. Water:

Holding a large amount of water in its leaves, Aloe Vera is very resistant to heat. However, careful irrigation is necessary in hot, dry weather to ensure growth. to ensure its growth. Excessive watering is harmful to the plant, which will rot, it is therefore essential to carry out an efficient drainage to prevent the roots from rotting.

Water that is too cold can be harmful to this plant, so water at room temperature is recommended room temperature is recommended. In all cases, irrigation should be moderate and stopped during the winter period.

Aloe Vera can withstand low rainfall (less than 500 millimeters per year) as well as high (500 to 2000 millimeters per year) (Grindlay R., 1986)

d. Temperature:

This plant of warm semi-tropical climates withstands large seasonal and daily temperature changes (Burte, et al., 1992), is recognized as the most resistant plant in the world. As long as the soil is not frozen, its roots can survive in freezing air. The leaves start to be affected when temperatures fall below 5°C. In contrast, Aloe Vera thrives at temperatures of 40°C and well above; it can survive the most extreme conditions of drought (Hennessee, et al., 1989).

1.8.2. Harvesting:

It takes about 3 years to harvest the Aloe Vera plants so that they are the right size. The leaves, on the other hand, can be harvested for about 7 years.

1.9. Chemical composition of Aloe Vera:

The plant is rich in a number of natural health-promoting substances. The raw pulp of Aloe Vera contains about 98.5% water, while the gel contains about 99.5% water. The remaining 0.5 to 1% contains about 250 active ingredients. Here is a quick overview of some of the most important components of Aloe Vera (ESHUN, et al., 2004)

1.9.1. Aloe Vera gel:

Fresh aloe Vera gel is an anti-bacterial and anti-fungal agent that can easily destroy micro-organisms and cleanse the body of poisons. It can also strengthen your immune system and speed up your metabolism (figure 9).

The main characteristics of the gel are (Morin, 2008):

- Viscous appearance.
- The absence of color, transparent.
- Its slightly bitter taste.

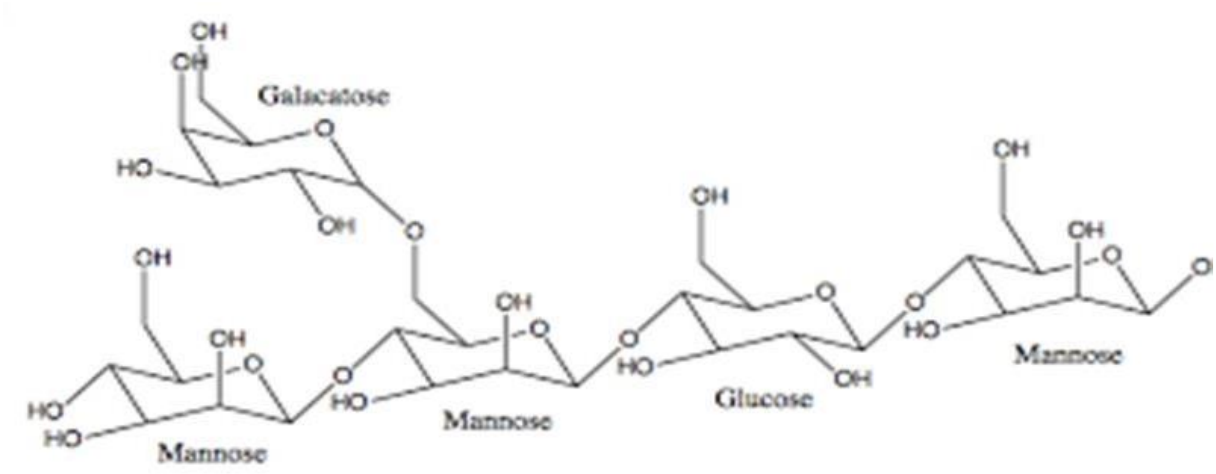


Figure 9: The chemical structure of Aloe Vera gel (sorrian, 2016)

a. Vitamins:

- Vitamin A (carotene): Improves vision, promotes skin and bone health, and protects cells from free radicals, Cells from free radicals.
- Vitamin B1 (thiamine): Necessary for tissue growth and energy production.
- Vitamin B2 (riboflavin): Common action with vitamin B6 for blood formation.
- Vitamin B3 (niacin): Helps regulate metabolism.

- Vitamin B6 (pyridoxine): Common action with vitamin B2 for blood formation.
- Vitamin B9 (folic acid): Ant-anemic, promotes the regeneration of red blood cells.
- Vitamin B12 (Cyanocobalamin): Essential for the metabolism, energy factor for the body's the body's nutritional functions and promotes the formation of red blood cells.
- Vitamin C (ascorbic acid): In combination with vitamin E, fights infection by immune system, promotes healing and maintains healthy skin.
- Vitamin E (tocopherol): Together with vitamin C, protects the cell membrane and helps to fight and heal infections (Natacha, 2013)

b. Enzymes :

- Amylase: Catalyzes the hydrolysis of starch into dextrin and then maltose.
- Bradykinase: Stimulates the immune system, analgesic, anti inflammation.
- Catalase: Prevents water accumulation in the body.
- Cellulase: Helps to digest cellulose.
- Creatine phosphoric (Muscle enzyme).
- Lipase: Aids digestion.
- Nucleotidase: Catalyzes the hydrolysis of nucleotides into nucleosides.
- Acid phosphatase: Marker of prostate cancer.
- Alkaline phosphatase: Regulates liver functions.
- Proteolytiase (or protease): Hydrolyses proteins within their constituents.
- Caprylic acid: is used in the treatment of fungal infections. (Surjushe, et al., 2008).

c. Minerals:

Aloe Vera is a magical plant that contains more than 20 different minerals, all of which are essential to the human body.

The human body, such as:

- Calcium: Formation of teeth and bones, muscle contraction and heart health.
- Magnesium: In combination with manganese, maintains the proper functioning of the nervous system and muscles.
- Chlorine: Antiseptic and disinfectant.
- Zinc: Accelerates healing, helps to maintain healthy teeth, bones and skin, and stimulates the immune system and the activity of the immune system and protein activity in healing.
- Manganese: Activates enzymes, strengthens bones, nerves and tissue.
- Chromium: Helps in protein metabolism, facilitates regulation of blood sugar levels and blood sugar levels and the circulatory system.

d. Additional minerals found in Aloe Vera include:

- Copper: A trace mineral essential to the balance of the body, and the formation of blood.
- Iron: Provides oxygen to red blood cells and promotes resistance to infection.
- Phosphorus: Bone growth, in association with calcium.
- Potassium: Regulates the fluid components of blood and muscles.
- Sodium: With potassium, maintains water balance levels in the body, transports amino acids and glucose to the cells.

e. Anthraquinones:

They are phenolic compounds with laxative, analgesic and antimicrobial effects lignin penetrates easily into the skin. Saponins are both depurative and antiseptic. The anthraquinones have analgesic and laxative properties:

Barbaloin (Antibiotic and cathartic), Isobarbaloin (Analgesic and antibiotic), Anthranol (Oxygen binding), Anthracene (Oxygen binding), Aloetic acid (Antibiotic), Aloe Emodin (Bactericide and laxative), Cinnamic acid(Detergent, germicide and fungicide), Cinnamic acid ester Ethereal oil (Analgesic and anaesthetic), Chrysophanic acid (Fungicide) (Skin fungus); Aloe ulcin (Blocks gastric secretions by reaction with histamine), Resestanole (Anti-inflammatory and bactericidal action) (would act as a natural corticoid) (Bazeeb, 2002) (Yimei Jia, 2008).

f. Amino acids:

Aloe contains 20 different amino acids, including seven of the eight essential amino acids

Amino acids, a group of proteins, play a role in all the functions of the body the body: providing energy, participating in brain functions (including emotional ones)

Amino acids, a set of proteins, play a role in all of the body's functions: providing energy, participating in brain functions (including emotional ones), intervening in tissue regeneration, etc.

"Essential" means that the body is not able to produce them itself...7 of the 8 "essential" amino acids are present in Aloe Vera, as are 11 of the 14 "essential" amino acids. of the 14 so-called "secondary" amino acids, which our body synthesizes from the 8 essential amino acids.

Essential amino acids: Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine.

Secondary amino acids: Aspartic acid, glutamic acid, alanine, arginine, cystine, glycine, histidine, hydrox proline, proline, serine, tyrosine (Bazeeb, 2002) (Yimei Jia, 2008).

1.10. Uses of the plant:

1.10.1. Food uses:

The food and beverage market is a promising area for Aloe Vera. It has been used as a resource for functional foods such as yoghurt or for the preparation of health drinks, including tea (Gage, 1996).

Aloe Vera does not appear to affect food taste or appearance, so it seems to be promise as a safe, natural and environmentally friendly alternative solution to conventional synthetic preservatives; it is well known that botanicals are widely used as a food supplement for health promotion or disease prevention (Serrano, 2006).

Aloe Vera gel can be used as an effective covering to prolong the quality and safety of fresh produce. Table grapes coated with Aloe Vera gel have significantly delayed the loss of functional compounds such as total phenolic acid and ascorbic acid. This is because Aloe Vera inhibits the growth of micro-organisms responsible for food-borne illnesses in humans and animals and food spoilage (Klein, 1988) (ESHUN, et al., 2004).

1.10.2. Cosmetic uses:

Generally, Aloe Vera has many uses both for humans and animals. Three distinct preparations of the plant are used: Aloe Vera latex, Aloe Vera gel and Aloe vera whole leaf extract, whose biological ingredients may act alone or in synergy (Boudreau, et al., 2004) (Davis, 1991).

The use of Aloe Vera in cosmetics is not new. Aloe Vera is used in concentrations ranging from 1 to 98%. It is well known that Aloe Vera gel retains moisture for extremely long periods and has soothing effects. Thus, Aloe Vera has found wide application in the cosmetic and toiletry industries, such as moisturizers, cleansers, sun lotions, toothpastes, mouthwashes, shaving creams, deodorants and shampoos (Christaki, 2010).

1.10.3. Medicinal uses:

The great healing capacity of Aloe Vera is to find a number of MPS present between 10,000-20,000 MPS per liter 8. In addition, the effects of Aloe Vera are in the treatment of scar tissue and the prevention of scar formation after a skin injury, probably are attributed to the activity of the amino acids necessary for the formation of new cells and due to the ability of its enzymes to promote the regeneration of the deepest layers of the skin (ESHUN, et al., 2004) (Choi, 2003).

a. Antibacterial and antifungal activity:

Many researchers have mentioned that Aloe Vera inhibits the growth of certain microorganisms such as Streptomycin pyogenes, Shigella flexneri, Klebsiella sp., especially against Gram-positive bacteria causing food poisoning or infections. As for the antifungal activity, it has received less attention, although an inhibitory activity against Candida has been reported (Christaki, 2010).

Aloe Vera is renowned in traditional medicine for its soothing and antimicrobial benefits. The antimicrobial activity has therefore often been tested in numerous scientific studies. It has been shown that the anthraquinones present in Aloe Vera latex are highly antimicrobial. When extracted with an ethanolic compound, they are effective against Gram minus bacteria (*Escherichia coli, Pseudomonas aeruginosa*) but also against Gram plus bacteria (*Staphylococcus aureus*). However, these compounds are very often removed from Aloe vera gel because of their properties, colour and toxicity, so they are very rarely present in cosmetic products (Pandey, et al., 2010).

Another component, this time present in Aloe Vera gel, has been characterized by its antimicrobial activity, fumaric acid. It has been tested and shown to be effective against four common bacteria: *Staphylococcus aureus, Streptococcus, Escherichia coli and Salmonella*.

It is therefore effective against both gram plus and gram minus bacteria. This acid, which is well known and used as a food preservative, is therefore present in the gel and is one of the compounds that give it an antimicrobial action (Chang, et al., 2011).

Since bacterial resistance is a major concern today, further studies have been carried out to isolate four other components that are effective against bacteria: pyrocatechol, cinnamic acid, coumaric acid and ascorbic acid. Aloe vera is therefore still far from being fully characterised and other compounds of interest could still be discovered (Lawrence, et al., 2009).

The antifungal activity of Aloe vera has also been tested in different studies; it has been shown to be effective against different types of fungi, including Candida albicans and Trichophyton rubrum (Jia, et al., 2008).

b. Antioxidant activity:

Plant derived antioxidants, e.g.; Phenolics, are known to be very important components due to their potential beneficial actions. Some antioxidant components are naturally present in the aqueous extract of Aloe Vera leaves which include polyphenols, flavonoids, ascorbic acid, β-carotene and α-tocopherol. The antioxidant activity of Aloe Vera barbadensis Miler leaf extracts obtained using four extraction solvents had good DPPH scavenging property. A significant increase in the levels of reduced glutathione, superoxide dismutase, glutathione

peroxidase and glutathione-S-transferase was also observed in the liver and kidney of treated rats (Moniruzzaman, 2012).

c. Anti-inflammatory activity:

The anti-inflammatory activity of mannose-6-phosphate is thought to resemble the effects observed for acetylated mannan in Aloe Vera gel, which reduces inflammation induced by inflammation via the promotion of prostaglandin synthesis and increased leukocyte infiltration. The effects of aqueous and ethanolic extracts of Aloe vera gel were tested on rat paw oedema and carrageenan-induced neutrophil migration. It was reported that the aqueous extracts inhibited oedema formation and decreased the number of migrating neutrophils. The ethanolic extract showed no effect on oedema, but reduced the number of migrating neutrophils (Hamman, 2008).

d. Anti-cancer effects:

The two fractions of Aloe Vera that are claimed to have anti-cancer effects include glycoproteins (lectins) and polysaccharides. The anti-tumor activity of polysaccharides isolated from Aloe Vera and specifically acemannan has been studied in numerous in vitro models as well as in different animal models. Various studies indicate anti-tumor activity for Aloe Vera gel in terms of reduction of tumor burden, tumor shrinkage, tumor necrosis and prolonged survival rate. One mechanism of action that has been proposed for these anti-cancer effects of polysaccharides is the stimulation of the immune response (Hamman, 2008).

e. Protection from skin exposure to UV and gamma rays:

Aloe Vera gel has been reported to have a protective effect against radiation damage to the skin. The exact role is not known, but after administration of Aloe Vera gel, an antioxidant protein, metallothionein, is generated in the skin, which clears hydroxyl radicals and prevents the suppression of superoxide dismutase and glutathione peroxidase in the skin. It reduces the production and release of skin keratinocyte-derived immunosuppressive cytokines such as interleukin 10 and therefore prevents UV-induced suppression of delayed hypersensitivity (Kumar, 2014).

f. Other effects:

Other effects that have been attributed to fresh Aloe vera gel include its healing effects in superficial wounds and traumas of the skin. Also, the reduction of pain at the site of the trauma is visible after taking this medicine (Henry, 1979).

The effects of aloe gel on the skin also improve the skin absorption of medicines. In a study on the effect of Aloe Vera on caffeine, colchicine, mefenamic acid, oxybutynin and kinin drugs, this effect of increased skin consumption (stratum) was observed (Cole, et al., 2007).

Aloe Vera (or yellow aloe) plant resembles a cactus and is a succulent, watery plant, whose leaves comprise mucilage (gel) tissue. This mucilage consists of some glycoprotein, which prevent inflation and pain and accelerate their tendency to improve. It also contains polysaccharides, which stimulate the growth and healing of the skin. The mucilage of this plant can be used for the treatment of internal and external wounds (ESHUN, et al., 2004) (Boudreau, et al., 2004).

1.10.4. Secondary effects of Aloe Vera:

Aloe Vera has some side effects in some situations. Sometimes, some people develop a mild allergic reaction marked by rash or itching, when using it as a topical treatment. Internal use of Aloe Vera latex can also cause abdominal pain or cramps or even diarrhea when products containing anthraquinones are consumed. Due to improper processing, Aloe Vera juice sometimes contains small amounts of the laxative compound in the latex. Due to its laxative effects, overuse can cause electrolyte imbalances,Electrolyte imbalances. Pregnant women should not take aloe latex as it can cause uterine contractions and trigger miscarriage. Aloe latex is not recommended for people with gastrointestinal disease, intestinal obstruction, appendicitis or stomach pain (Roos, 2010).

Since ancient times, the human has used the Aloe Vera plant in many uses including food, decoration and treatment, and ancient man used the plant without knowing its chemical benefits and the advances in science and discovery. Man has become able to identify the active chemical substances that exist.

Among these; the polyphenols.....

CHAPTER II

POLYPHENOLS

2.1. Generality of secondary metabolites:

Secondary metabolites are complex organic molecules synthesized by autotrophic plants. The products of secondary metabolism are very numerous, with more than 200,000 defined structures. They are divided mainly into three large families: polyphenols, trepans and alcaloids (Boudjouref, 2011) (Hartmann, 2007) (Abderrazak, 2007).

2.1.1Polyphenols:

Phenolic compounds or polyphenols (PPs) are widely distributed products of secondary plant metabolism with several phenolic groups, with or without other functions and with at least 9000 different known structures, they are an integral part of human and animal nutrition and are considered to be almost universal compounds of plants. Structurally, they are split into several classes, ranging from compounds with a simple phenolic core to complex polymeric compounds such as tannins. Polyphenols are the active ingredients of many medicinal plants. They are generally found in all vascular plants, where they can be located in various organ (Bahorun, 1997) (Martin, 2002) (Collin, 2011).

These elements play a fundamental role as they are important elements of the sensory (colour and organoleptic characteristics) and nutritional qualities of plants, such as vegetables, fruit, cereals or dried fruit, as well as in beverages, coffee, cocoa or tea. It is estimated that humans consume about one gram of polyphenols each day, which is ten times more than vitamin C and 100 times more than carotenoids or vitamin E (Scalbert, et al., 2005).

2.1.2. Flavonoids:

Flavonoids are almost universal products of plants, often responsible for certain colouring of many plants. All flavonoids have the same basic structure, the flavan ring consisting of 15 carbon atoms which are assembled in 3 rings: A, B and C (A and B) are aromatic rings, and C is a central oxygenated heterocycle). heterocycle). Flavonoids are often found in fruits and vegetables (Bravo, 1998).

Depending on the degree of oxidation of the central heterocycle, flavonoids are divided into several classes: flavones, flavonols, flavonones, anthocyanins and isoflavones (Li, 2007).

2.2. Polyphenols inside the plant:

2.2.1. Location and function:

At the cell level, phenolic compounds are mainly distributed in two compartments: the vacuoles and the cell wall. In the vacuoles, the polyphenols are conjugated with sugars or an organic acid, which increase their solubility and limits their toxicity for the cell, At the wall level, lignin and flavonoids are mainly found in the parietal structures (Bénard, 2009).

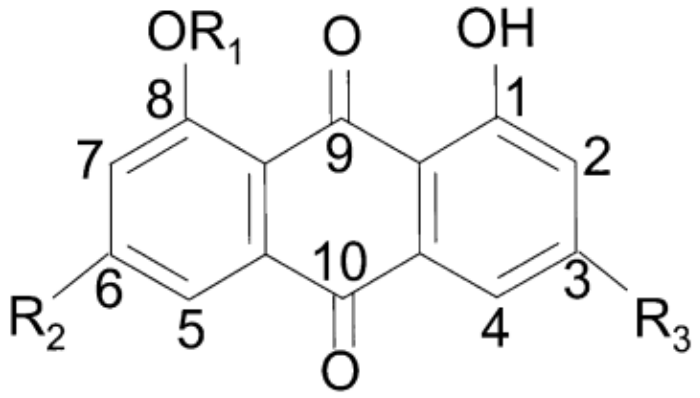
At tissue level, the location of polyphenols is linked to their role in the plant and can be very characteristic. Within the leaves themselves, the distribution of the compounds is variable, for example the majority of anthocyanins and flavonoids are present in the epidermis (Tomas, et al., 2010).

2.2.2. Mechanism effect of the Emodin:

Emodin: chemical structure and distribution:

Emodin belongs to the anthraquinones, a group of > 170 natural compounds that make up the largest group of natural quinones (Thomson, 1997). Among the most common naturally occurring anthraquinones aglycones in higher plants are emodin, rhein, chrysophanol, aloe-emodin, and physcion (Harborne, et al., 1999). More than half of the natural anthraquinones are found in lower fungi, particularly in Penicillium and Aspergillus species, and in lichens. Others are found in higher plants and in isolated instances, in insects (Evans, 1996). Anthraquinone may either be formed via the acetate-malonate pathway in Polygonaceae and Rhamnaceae or O-succinylbenzoic acid in the Bignoniaceae and Verbenaceae (Evans, 1996) (Harborne, et al., 1999). The basic chemical structure of anthraquinone is an anthracene ring (tricyclic aromatic) with two ketone groups in position C9 and C10 ,In plants, anthraquinones are mostly present as sugar derivatives – the glycosides – but the free form – the aglycones – are widely distributed as well (Evans, 1996) (Thomson, 1997). Several biochemical pathways that transform one anthraquinone to another have been discovered. For example, chrysophanol is synthesized in plants by dehydroxylation of emodin, an enzymatic conversion that is mediated by NADPH as cofactor (Anderson, et al., 1988). It was also suggested that physion is derived from emodin (Thomson, 1997). The anthraquinone glycosides formed when one or more sugar molecules, mostly glucose or rhamnose, are bound to the aglycone by a β-glycoside linkage to hydroxyl group at position C8 (in the case of glucose) or the one at C6 (in the case of rhamnose) (Dewick, 1998). Among the most common emodin-related

glycosides are emodin-8-glucose, frangulin and glucofrangulin (figure 10) (Harborne, et al., 1999).



R_1	R_2	R_3	Compound
aglycones			
H	OH	CH_3	emodin
H	H	CH_3	chrysophanol
H	OCH_3	CH_3	physcion
H	H	CH_2OH	aloe-emodin
H	H	COOH	rhein
glycosides			
Glucose	OH	CH_3	emodin-8-glucose
H	O-Rhamnose	CH_3	frangulin A
Glucose	O-Rhamnose	CH_3	glucofrangulin A

Figure 10: Structural formula of emodin and some other anthraquinone aglycones and glycosides present in high plants.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone is mainly reported in three plant families: Fabaceae (Cassia spp.), Polygonaceae (Rheum, Rumex and Polygonum spp.) and Rhamnaceae (Rhamnus and Ventilago spp.) . It seems that this is not a function of the larger number of species assayed in these families relative to other families but that emodin is actually more common among species of these three families. However, a comprehensive literature survey revealed that emodin has already been identified in at least 17 plant families, 28 genera and 94 species. Interesting records of emodin have been documented recently in Asteraceae, Poaceae and Simaroubaceae, among others .The presence of emodin in Aloe (Liliaceae) is rather controversial (Dange, 1996), and it might be rare in this family. Emodin has a worldwide distribution, occurring in subtropical and tropical families (e.g. Bignoniaceae and Simaroubaceae), in families that mainly inhabit the temperate region (e.g. Polygonaceae and Saxifragaceae), and in families inhabiting both the tropics and the

temperate regions (e.g. Rhamnaceae and Clusiaceae). Furthermore, emodin occurs in diverse life forms including trees, shrubs, lianas and herbs. (Dange, 1996).

CHAPTER III

BACTERIAL STRAINS

3. Commensal micro-organisms:

3.1. The bacteria:

Bacteria are ubiquitous and are present in all types of biotopes encountered on earth they can be isolated from soil, water, air, skin and especially in the intestines of animals Such as *pseudomonas, salmonella, streptococcus, staphylococcus, Escherichia coli* (Fredrickson, et al., 2004).

3.2. Staphylococcus:

Staphylococcus aureus is the most pathogenic species of the genus Staphylococcus it appears as a clustered, Gram-positive shell its carotenoid content gives it a golden color which gives its name (Françoise, et al., 2010).

3.2.1. Habitats:

Staphylococci are ubiquitous germs that can live:

- As commensal bacteria on the skin and mucous membranes of humans and animals.
- As pathogenic bacteria that cause human or animal infections, which can be very dangerous.

3.2.2. Classification of Bergey (1994 :

Embranchement	firmicutes
Groupe 17	cocci gram +
Ex famille des micrococcaceae	famille des micrococcaceae
Genres staphylococcus	micrococcus,ex-micrococcus (kocuria, Kytococcus,dermacoccus)

3.2.3. Pathogenicity:

Staphylococcus aureus, a species of coagulase-positive staphylococcus, is frequently encountered in humans and can be responsible for various infections (skin, nosocomial, food poisoning).

3.3. Escherichia coli:

Escherichia coli are the type species of the genus *Escherichia*, commonly known as (E. coli). This species, which has been the subject of a large number of studies, is the model for aerobic Gram-negative bacilli. (Barnard, et al., 2002).

3.3.1. Habitats:

Escherichia coli are a commensal bacterium of the colon in humans and animals;

In humans, *E coli* are the dominant species of the aerobic bacterial flora of the colon. They are also present but at a lower level in the small intestine. (Pillet, et al., 1986).

3.3.2. Scientific classification: (Pillet, et al., 1986)

Kingdom: Bateria

Branch: Proteobacteria

Class: Gamma proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: Escherichia

Species: Coli

3.3.3. Pathogenicity:

E coli can cause gastroenteritis with variable clinical manifestations: allergy diarrheas, bloody diarrheas, coliform diarrheas; it can also cause meningitis infections or septicaemia (Olin, 2000).

3.4. Pseudomonas:

Pseudomonas aeruginosa, otherwise known as the pyocyanin bacillus, is a gram-negative bacterium of the genus Pseudomonas. Devoid of spores and capsules, it is a saprophytic bacterium of the air, water and soil, commensal of all the integuments and mucous membranes of humans and animals, and has a broad pathogenic power. (BedouiH, et al., 2006).

The species *P. aeruginosa* is very abundant in our environment and can be found in soil, water, on the surface of plants and animals. In hospitals, *P. aeruginosa* is sometimes found in aseptic solutions and on instruments such as catheters, probes, or in pipes and sinks. Its enormous capacity to adapt to various environments is certainly linked to the plasticity of its large genome (about 6Mpb) (Wolfgang, et al., 2003).

3.4.1. Classification: (migula, 1900)

Kingdom: Bacteria

Division: Proteobacteria

Class: Gammaproteobacteria

Order: Peudomonadales

Family: Pseudomonadaceae

Genus: Pseudomonas

3.4.2. Pathogenicity:

The pyocyanine bacillus is basically a phylogenic bacterium responsible for the formation of surface and deep suppurations in humans and animals, from which it can be isolated (BedouiH, et al., 2006).

CHAPTER IV

MATERIALS AND METHODS

4. Materials and Methods:

Presentation of the location of the experimental study:

My experimental study was conducted at the laboratory of biochemistry and microbiology at the University Abdelhamid Ibn Badis, Mostaganem.

Work objective:

The present study aims particularly to proceed to the extraction of polyphynol from Aloe vera, especially from the aloin and phenolic compounds of the latex by the use of Annok method. In order to study the antibacterial activity of these different PPs pure and at different dilutions on gram negative pathogens, Pseudomonas aeruginosa, Escherichia coli, and gram positive bacteria, Staphylococcus aureus.

4.1. Materials:

4.1.1. Treatments of the plant material:

My study focused on Aloe Vera plant: Aloe Barbadensis Miller (figure 11). The parts of interest for our study for the plants were the fresh leaves, harvested in the state of Mostaganem(Ain tadles).24-04-2021.

Figure 11: Plant of aloe Vera (Aloe Barbadensis Miller).

4.1.2. Strains studied:

We selected 3 pathogenic strains (figure 12), which are often responsible for major public health problems, and have developed antibiotic resistance.

We selected 2 groups of bacteria:

- Gram negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*.

-Gram-positive bacteria: *staphylococcus aureus.*

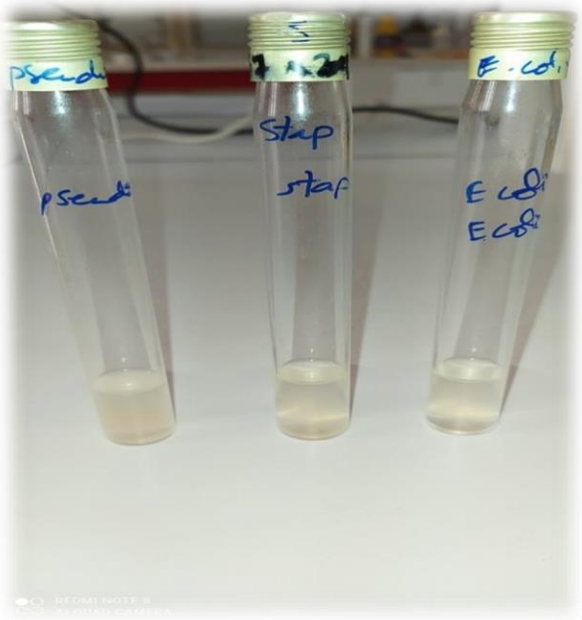


Figure 12: Bacterial strains

4.2. Methods:

4.2.1. Extraction method:

The extraction was carried out according to the method of Annok (figure 13) which consists in cleaning, cutting and grinding (Moulin) 100 g of Aloe Vera to which 125 ml of pure methanol 99% is added before leaving the mixture under agitation for 24 h at room temperature and in the dark. The crude extract was then filtered through Whatman N°4 paper, then the residue was recovered in another vessel with 125 ml of 99% ethyl acetate and left under stirring for 30 min then the suspensions were filtered again through a Whatman N°4 paper. The two filtrates obtained were mixed to be dried by evaporation of the solvent at 45°C with a rot vapor (figure 13) (Arnnok, et al., 2012).

4.2.1.1. Experimental protocol for the aloe Vera extract:

The same as the annonk method (figure 13) and do a separate work for both gel and latex, and tram evry part of the plant with separate method the latex treat it with methanol and the gel treat it with ethyl acetate just the same as annonk method (figure 13).

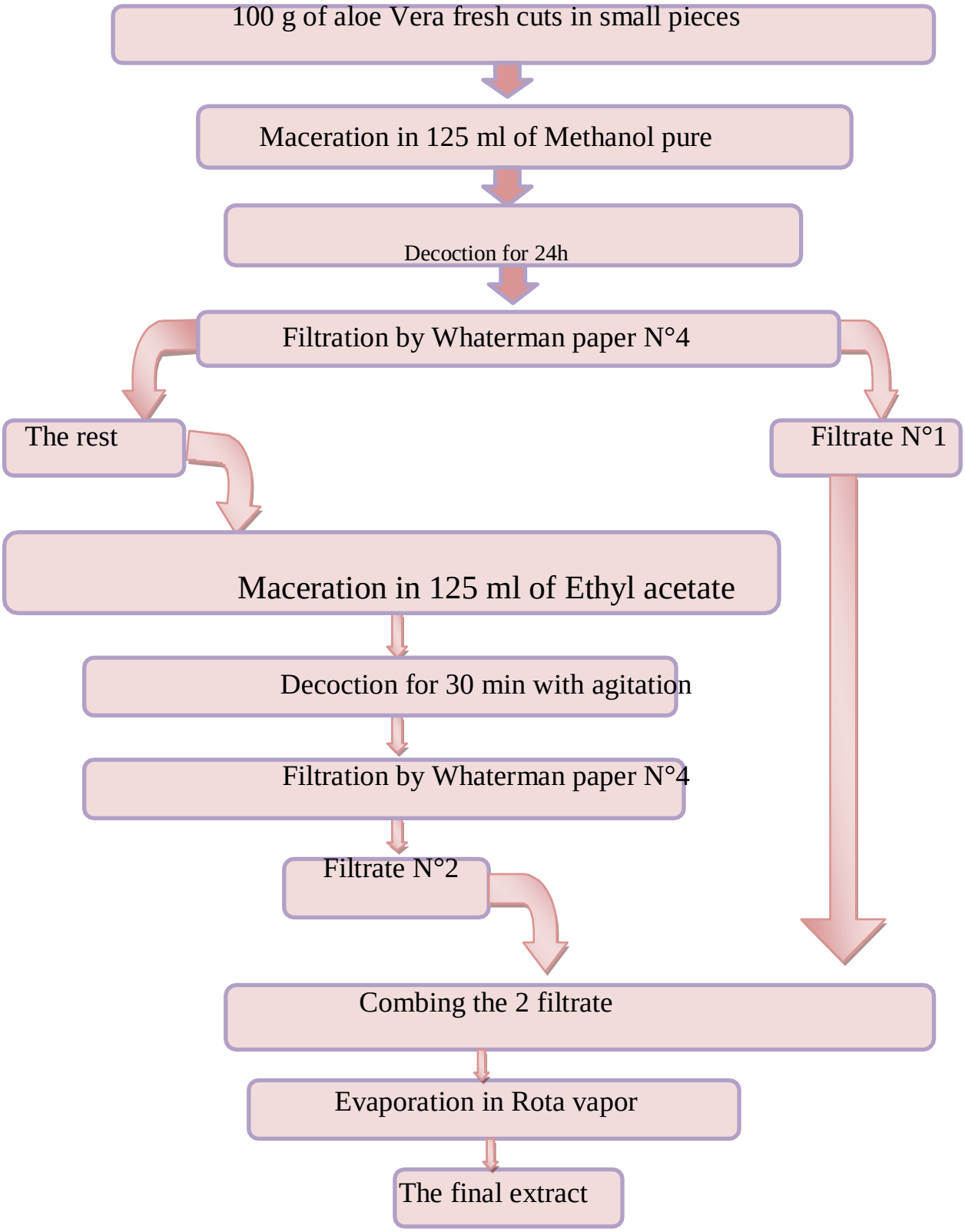


Figure 13: annonk method (Arnnok, et al., 2012).

4.2.1.2. Dilution method:

This technique demonstrates the effects of the antimicrobial activity of PP's after a number of dilutions.

4.2.1.3. Preparation of dilutions:

0.5 ml of PP is aseptically introduced with a sterile graduated pipette into a sterile Eppendorf containing 0.5 ml of Dmso diluents. This preparation corresponds to the Dm which consists of a 1/2 or 50% dilution. The Dm is put in an oven at 37°C for 20 min with shaking of the eppendorf from time to time to homogenize the 2 phases of the medium.

4.2.2. Microbiological study:

4.2.2.1. Identification of pure strains:

Coloration of Gram:

In an aseptic area, prepare 1 smear of a gram + culture and 1 smear of a gram - culture (Delarras, 2007).

- Pour the Gentian violet on the smear and let it react for 30 seconds
- Drain the Gentian violet and rinse with distilled water
- Add a few drops of Lugol's and let it react for 1 minute
- Decolorize with alcohol
- Rinse the slide again with distilled water
- Pour the Fuchsin and let react for 20 seconds
- Rinse with distilled water and let dry over a Bunsen burner flame.
- Observe the smears under a light microscope with a x100 objective on immersion.

Bacteria that appear in purple are Gram positive, and those that appear in pink are gram negative.

4.2.2.2. Verification of the sensitivity of the target strains of our study to reference antibiotics:

In order to verify the possibility of development of any resistance by our studied pathogenic strains to the different widely used reference antibiotics prescribed for preventive purposes or as treatments. We proceeded to a preliminary sensitivity test of *E.Coli, P.aeruginosa, S.aureus* to ampicillin and to amoxicillin-clavulanic acid.

4.2.2.3. Preparation of the inoculums:

a. Pre-culture preparation:

The antibacterial method tests are performed with a sample of young cultures (18 to 24 hours) in exponential growth phase. The reactivation of the strains is performed by inoculation of the bacterial species in a liquid medium (nutrient broth). After incubating for 24 hours at 37°C.

b. Preparation of the bacterial suspension:

From young cultures on nutrient broth, put a bacterial suspension in a tube containing 7 ml of sterile nutrient broth, vortex for a few seconds.

Then calculate the optical density of the bacterial solution contained in physiological water knowing that the bacterial optical density is between 0.08 -0.1.

Before;

- The spectrophotometer must be adjusted to a frequency of 620nm.
- Then calculate the optical density of the physiological water contained in a cell as a control.

Then deposit another cell containing the bacterial solution contained in physiological water in order to calculate its optical density which will be included between 0,08-0,1.

Once the optical densities of the bacterial solution are adjusted in relation to the interval of 0.08 -0.1, we begin the seeding of these bacterial solutions contained in physiological water in the medium MH.

4.2.3. Choice and performance of the antibiogram:

In order to test the sensitivity of different strains of pathogenic bacteria selected in our study to the PP's, we adopted the antibiogram on an MH agar medium, which consists of placing disks soaked with a volume of 2 ul of our PP's, pure and diluted to 50% in DMSO on the agar previously seeded by a culture in the nutrient broth of the studied pure strains.

a. Seeding:

Inoculation should be done by spreading a drop of the bacterial suspension on the surface of the MH agar with a sterile swab

To do this, rub the swab over the entire dry surface of the agar, from top to bottom, forming tight ridges.

Repeat the same procedure twice, rotating the plate 60° each time, remembering to rotate the swab on itself.

Finish plating by rubbing the swab around the edge of the agar.

b. Preparation of discs:

The disks are prepared using Wattman paper № 4 by punching 2 holes in the paper, 5 mm in diameter. Then these disks are placed in a test tube, autoclaved, and stored at room temperature.

Take with sterile forceps the sterilized disks, the latter is soaked with PPs for 30s, then place in the Petri dish 04 disks soaked on the medium MH seeded.

Let the Petri dishes rest for 30 min until the discs are fixed on the solidified MH medium.

c. Incubation :

Incubate the Petri dishes in the oven at 37°c for 24 hours.

d. Reading the results:

The reading of the results, allowing estimating the sensitivity of the studied strains to the PPs of Aloe Vera is carried out by measuring the diameters of the zone of inhibition. These are measured with a ruler and expressed in (mm) (including the diameter of the 5 mm disk).

Sensitivity to the essential oil was classified according to the diameters of the inhibition halos on the following scale (PONCE, et al., 2003):

- Non-sensitive (-) or resistant: diameter < 8 mm.
- Sensitive (+): diameter between 9 and 14 mm.
- Very sensitive (++): diameter between 15 and 19 mm.
- Extremely sensitive (+++): diameter > 20 mm.

e. Statistical analyses:

Collected data in this randomized work were subjected to variance analysis (Cary, 2008). Duncan's multiple range tests was used to distinguish treatment means. A single degree of freedom contrasts was adopted to estimate the significant impacts of the thermal acclimation and the listed dietary supplementation. The level of $P<0.05$ was considered for significant.

RESULTS AND DISCUSSIONS

5. Results and discussions:

According to the results obtained by students of Abdelhamid Ibn Badis-Mostaganem University; during a similar graduation study of a Master in biology; the elimination method of Aloe Vera was effective against the bacteria such as the gram+ and the gram- bacteria.

The harvesting period, essential in terms of yield and quality of the PP, the climate, the geographical area, the genetics of the plant, the organ of the plant used, the degree of freshness, the extraction method used, etc. could explain this difference (Kelen M. & Tepe B).

5.1. Identification of strains:

Macroscopic observation of the colonies, after Gram staining of the isolated strains, allowed us to identify the bacteria in pink, confirming that they are Gram negative bacteria. These last ones are of *Coccobacillus* form for *E.Coli* (figure 14), and Bacillus form for *P aeruginosa*(13), and those in purple confirming us that they are indeed Gram positive bacteria of Cocus grape forms for the *S aeurues*(figure 15).

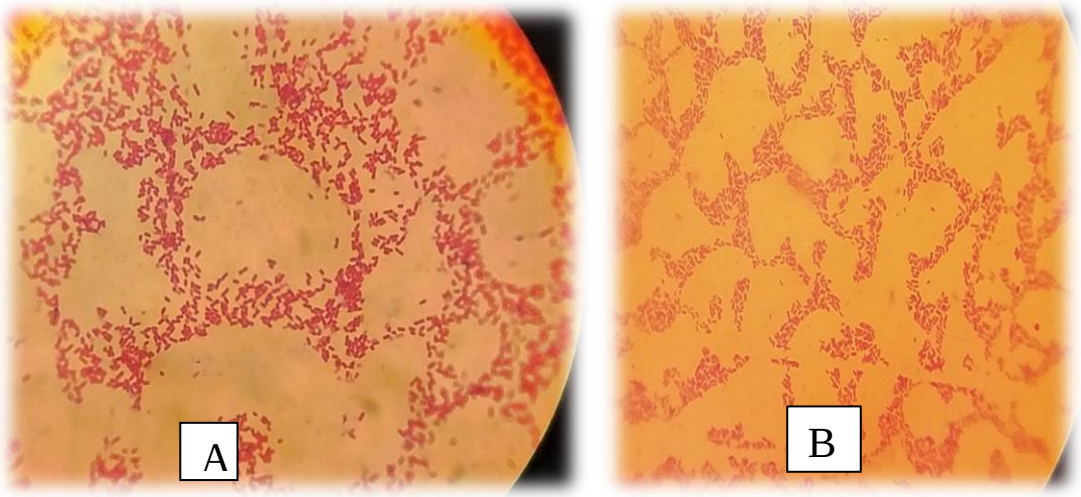


Figure 14: Coloration of Gram observation microscopy (X 100).

A: *Escherichia Coli*, B: *Pseudomonas aeruginosa*.

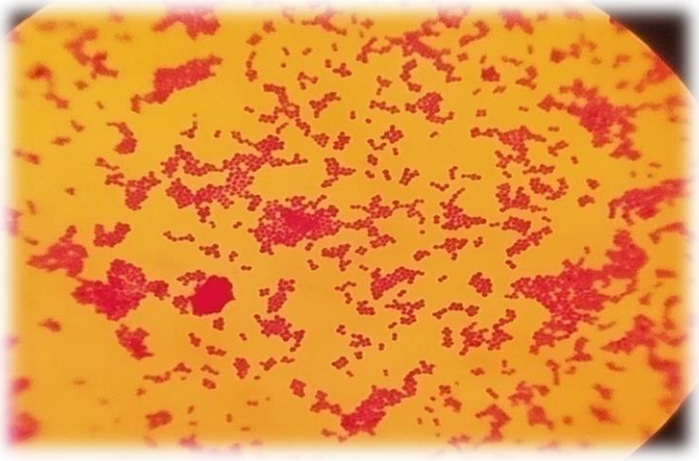


Figure 15: Coloration of Gram observation microscopy (X 100) of *Staphylococcus aureus.*

5.2.1. Verification of the sensitivity of the strains studied to the reference antibiotics:

5.2.1.1. Sensitivity to amoxicillin/clavulanic acid:

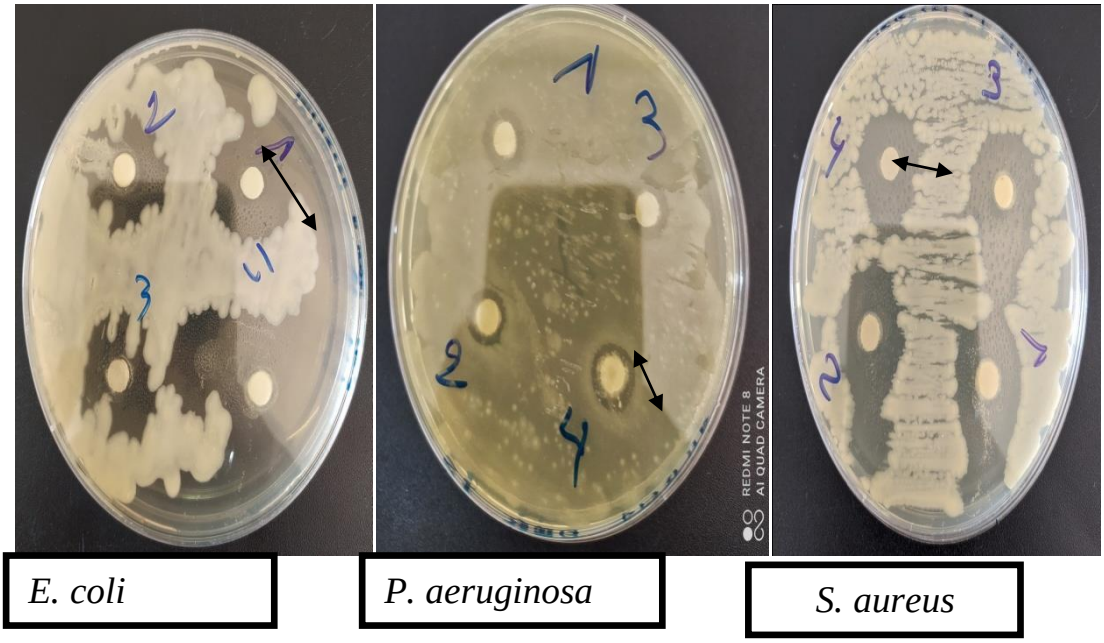


Figure 16: Susceptibility of strains to amoxicillin/clavulanic acid marked by zones of inhibition .

Table 1: Diameters of the inhibition zones in (mm). Marking the sensitivity of strains to amoxicillin/clavulanic acid marked by zones of inhibition.

The Strains	*E. coli*	*Ps. aeruginosa*	*S. aureus*
Diametere of inhibition (mm)	$18{,}16\pm3{,}97^{b}$	$11{,}5\pm3{,}17^{a}$	$17{,}5\pm2{,}71^{c}$
Sensitivity (±)	(++)	(+)	(++)

The diameters of the discs (5mm) are included in the determination of the inhibition zone diameters

(a,b,c ,d,e) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, results are expressed as means ± standard deviation

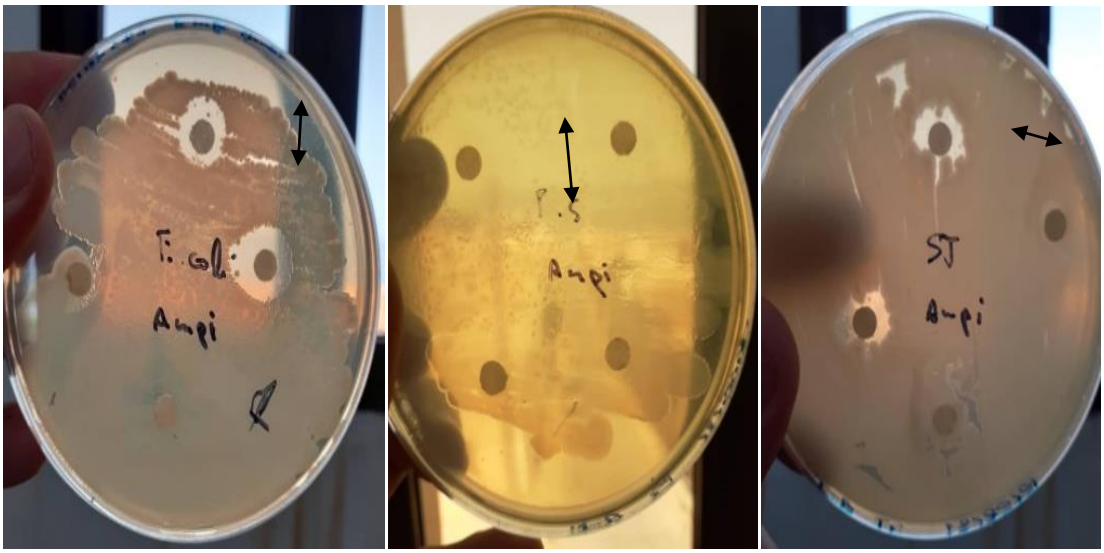
Sensitive (+), Very sensitive (++), Resistant (-), Extremely sensitive (+++)

The diameters of the zones of inhibition delimited by amoxicillin for the three strains were found to be very large in *E coli* 18.16±3.97significantly larger than that of *S aureus* 17.5±2.71. That of *P aeruginosa* 11.5±3.17 significantly lowers than the other strains.

Discussion:

For a reference antibiotic and despite the fact that the diameters of inhibition appear interesting from a theoretical point of view and in numerical figures, they remain far from the therapeutic expectations. It even seems that there is an ongoing regression of the sensitivity of the three strains studied to amoxicillin/clavulanic acid.

5.2.1.2. Ampicillin sensitivity:



E. coli | *P.aeruginosa* | *S. aureus*

Figure 17 : Susceptibility of strains to ampicillin marked by zones of inhibition.

Table 2 : Diameters of the inhibition zones in (mm). Marking the sensitivity of strains to amoxicillin/clavulanic acid marked by zones of inhibition.

The strains	*E. coli*	*Ps. aeruginosa*	*S. aureus*
Diametere of the inhibition (mm)	$10,75\pm2,49^{d}$	$9,08\pm3,26^{a}$	$8,5\pm2,50^{d}$
Sensibility	+	+	-

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diameters

(a,b,c ,d)) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, results are expressed as means ± standard deviation

Sensitive (+), Very sensitive (++), Resistant (-), Extremely sensitive (+++)

The diameters of inhibition zones are recorded larger for E. Coli10.75±2.49, followed by that of *Paeruginosa* 9.08±3.26, with a highly significant difference between the two values. On the other hand, the inhibition diameter labeled by ampicillin for S. aureus was 8.5±2.50 significantly lower than those labeled for *E coli* and *P aeruginosa*

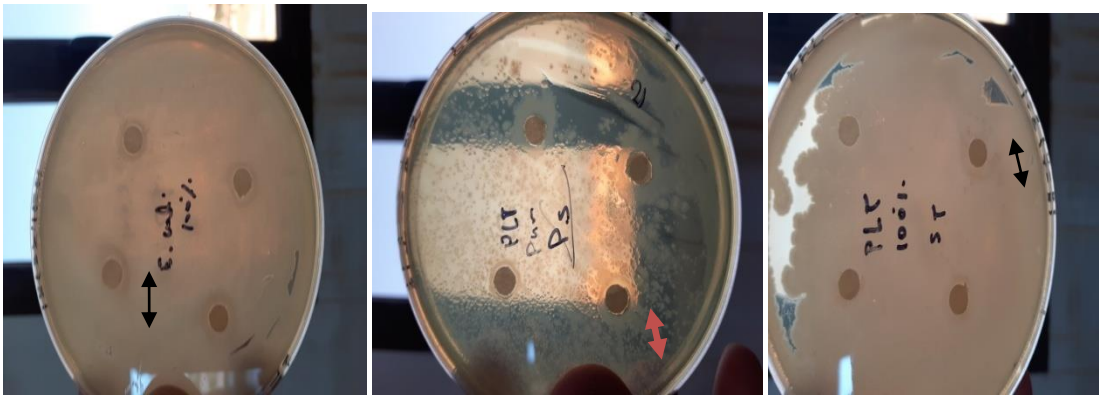
Discussion:

The results of the preliminary tests of the sensitivity of the target pathogenesis strains of our study; show us that these strains have developed a resistance to ampicillin which is very important in *S aureus*. We note that the sensitivity of the two other strains to ampicillin has decreased; announcing a development of a resistance in progress.

5.2.2. Antibacterial activity of pure polyphynols of Aloe Vera plant:

The effect of Polyphynols of the Aloe Vera (raw extract, the aloin pure and phynolic component of the latex) on the growth of the bacterial strains studied: *S aureus, P aeruginosa, E coli* (figure 18) in our experiment, are estimated by measuring the diameters of the inhibition zones expressed in mm. The values of the diameters are described in the tables and figures below:

a. Antibacterial effect of the pure raw extract:



E. coli | *Ps. aeruginosa* | *S. aureus*

Figure 18 : Sensitivity of strains to pure raw extract marked by zones of inhibition.

Table 3: Diameters of the inhibition zones in (mm). Marking the sensitivity of strains to pure raw extract marked by zones of inhibition.

The strains	*E. coli*	*P. aeruginosa*	*S. aureus*
Diametere of the inhibition (mm)	$10,25\pm1,545^{b}$	$7,25\pm3,793^{a}$	$10,25\pm1,685^{c}$
Sensibility (±)	(+)	(-)	(+)

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diameters

(a,b,c ,d,e) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, results are expressed as means ± standard deviation

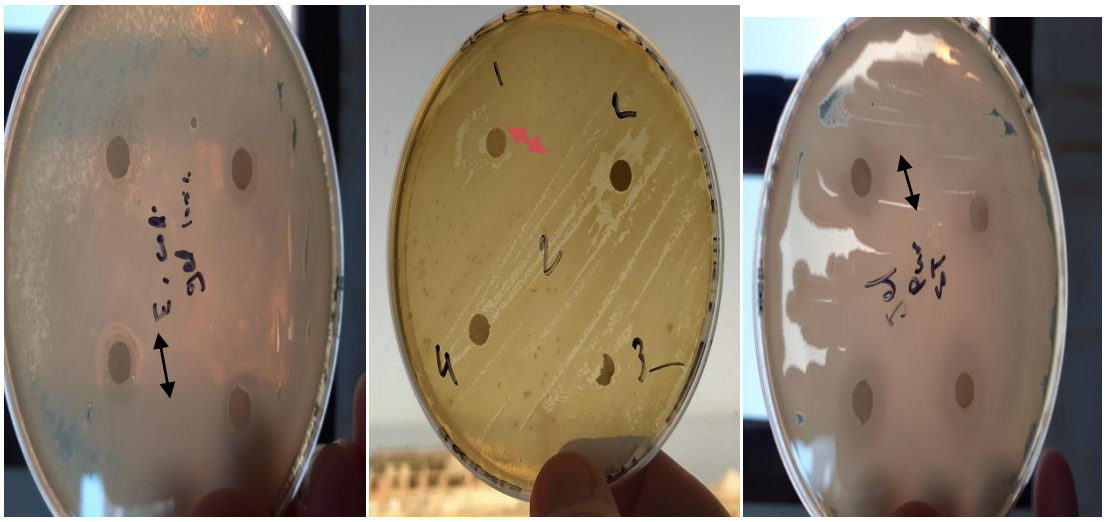
Sensitive (+), Very sensitive (++), Resistant (-), Extremely sensitive (+++)

We observe inhibition zone diameters considered marked by *E coli* and *S aureus* are similar 10,25±1,545 and 10,25±1,685 straight but they are significantly different, On the other hand, the diameter of inhibition noted for *P aeruginosa* 7.25 ± 3,793 significantly less compared to the diameter previously recorded for the other strains.

Discussion:

we observe that the stain of *P aeruginosa* gain against resistant against the raw extract and for the two others strains it show a sensibility compare to the antibacterial of reference it give almost the same result that showed on amoxicillin/clavulanic acid sensibility's and its seems that the stains starts development resist to raw extract.

b. Antibacterial effect of the pure Aloin:



E. coli | *Ps. aeruginosa* | *S. aureus*

Figure 19: Sensitivity of pure aloin strains marked by inhibition zones .

Table 4: Diameters of the inhibition zones in (mm). Marking the sensitivity of strains to pure aloin marked by zones of inhibition.

The strains	E. coli	P. Aeruginosa	S. aureus
Diameter of the inhibition (mm)	9,75±1,913[b]	3,5±4,503[c]	13,083±1,165[b]
Sensibility (±)	(+)	(-)	(+)

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diameters.

(a, b, c) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, the results are expressed as means ± standard deviation

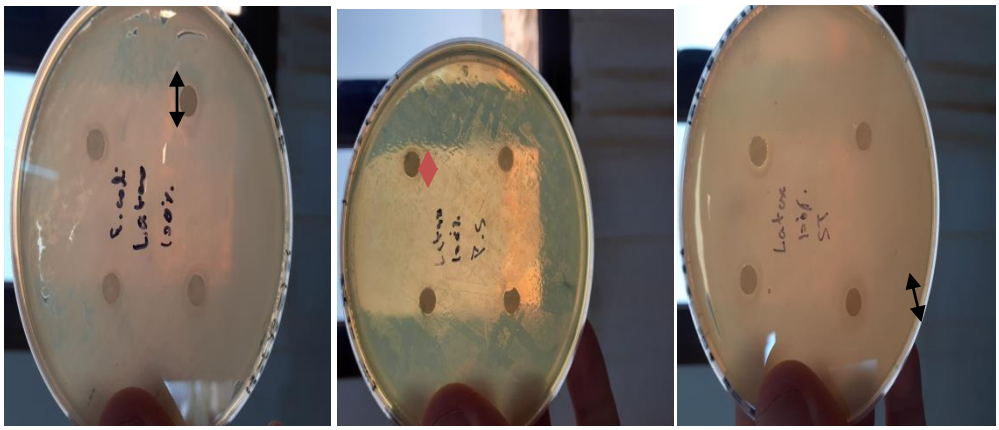
Sensitive (+), Very sensitive (++), Resistant (-), Extremely Sensitive (+++)

Diameters of the inhibition zones in the two strains are important in *S aureus* by 13,083±1,165 and we see a less diameter value in E coli compare to *S aureus* but they have no significant difference, for the *P aerginosa* show a less significant diameter to the other strains 3,5±4,503.

Discussion:

The *P aeruginosa* show a high resistant to the pure aloin and for the rest strains get sensibility against the pure aloin but if we compare it to the amoxicillin/clavulanic acid and ampicilin we can say that these are developing a resistant.

c. Antibacterial effect of the phynolic components of latex:



E. coli | *Ps. aeruginosa* | *S. aureus*

Figure 20 : Sensitivity of strains on phyolic component of latex pure marked by zones of inhibition.

Table 5 : Diameters of the inhibition zones in (mm). Marking the sensitivity of strains to the phinolic components of latex marked by zones of inhibition.

The strains	*E. coli*	*P. aeruginosa*	*S. aureus*
Diametere of the inhibition (mm)	9,417±1,443[b]	7,5±4,964[a]	10,333±1,67[c]
Sensibility (±)	(-)	(-)	(+)

The diameters of the discs (5mm) are included in the measurements of the Inhibition zone diameters

(a,b,c ,d,e) are homogeneous groups indicating a significant difference (P<0.05)

N = 3, results are expressed as means ± standard deviation

Sensitive (+), Very sensitive (++), Resistant (-), extremely sensitive (+++)

Diameter of the inhibition showed the high value in *S aureus* 10,333±1,67 followed by E coli 9,417±1,443 then the last *P aeruginosa* 7,5±4,964,and we see that these all three strains are significant differences.

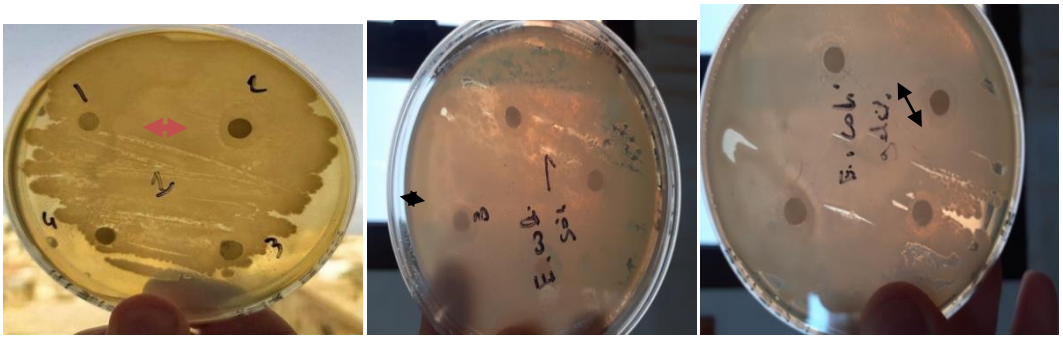
Discussion:

As we see most of the strains gain a resistant to the pure aloin unlike the S aureus, this strain get a sensibility against the pure aloin.

These result showed the opposite of what we see on the ampocillin resistant on *E coli* and *P aeruginosa* and sensitive on *S aureus* (table 2).

5.2.3. Comparison of the antibacterial effects of pure and diluted PPs with the effects of reference antibiotics:

a. Comparative study between the effects of pure and diluted PPs studied on *E.Coli* and reference antibiotics:



DMSO | Raw extract | Aloine 50%

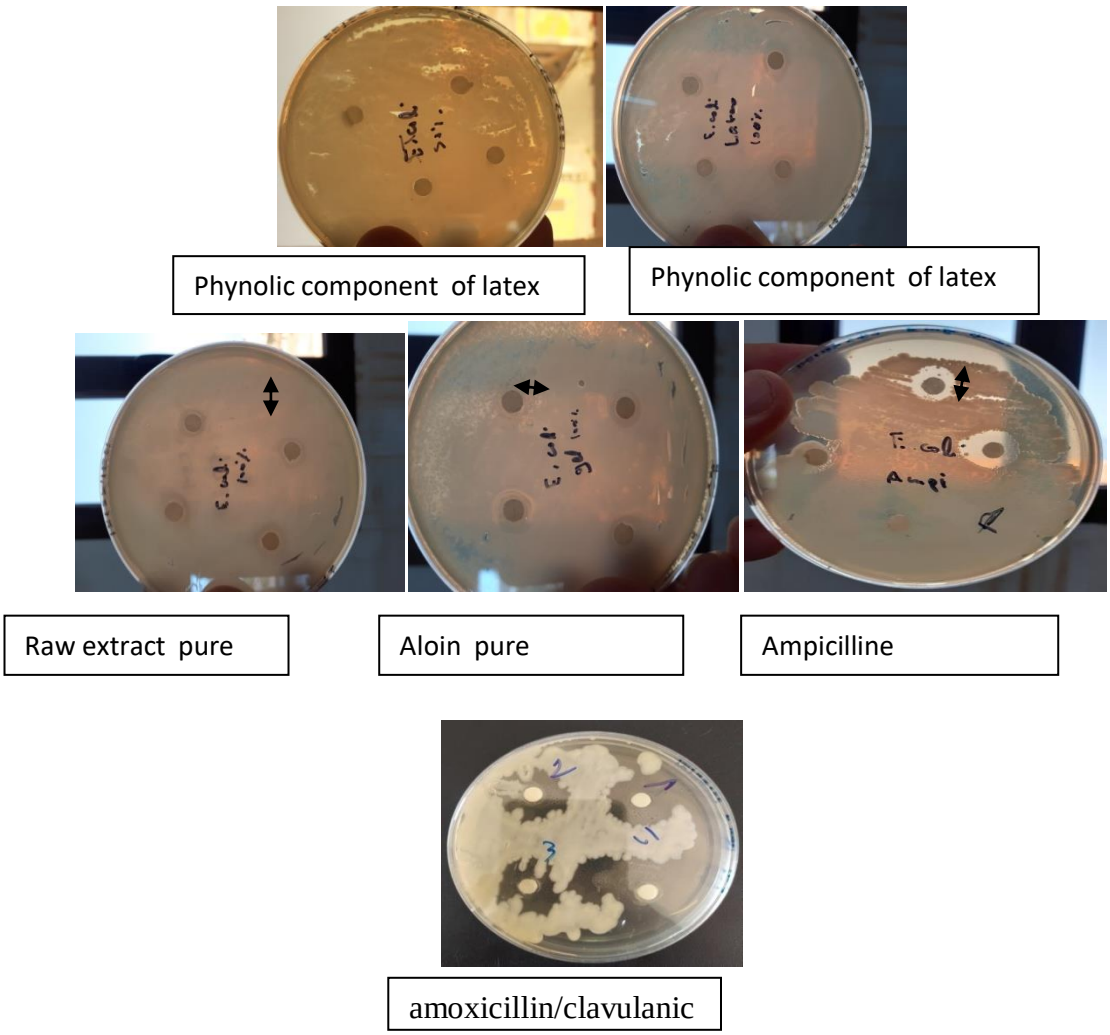


Figure 21 : compare of the sensitivity of *E coli* to pure and 50% diluted PPs of aloe vera and to reference antibiotics, marked by zones of inhibition.

Table 6: Diameters of the inhibition zones in (mm). Marking by the sensitivity of E. Coli to pure and diluted PPs at 50% of aloe Vera and to reference antibiotics.

	Raw extract	Raw extract 50%	Aloine pure	Aloine 50%	PCL pure	PCL 50%	Ampli	amoxicillin/clavulanic
E .coli	10,25± 1,545^{b}	10,917± 2,109^{b}	9,75±1 ,913^{b}	11,5±2 ,355^{b}	9,417± 1,443^{b}	10,917 ±1,24^{b}	18,167± 3,973^{a}	9,917±1,165^{b}
Sensi								

bility (±)	(+)	(+)	(+)	(+)	(+)	(+)	(++)	(+)

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diameters.

(a,b)are homogeneous groups indicating a significant difference (P<0.05)

N = 3, results are expressed as means ± standard deviation

Sensitive (+), Very sensitive (++), Resistant (-), Extremely sensitive (+++)

Diameters of the inhibition zones marking by the sensitivity of *E coli* starting from aloin 50% with high value 11,5±2,355 the minimum value in Phynolic component of latex pure with 9,417±1,443 and we see that these all molecules pure or diluted have that same significant value the same as amoxicillin/clavulanic acid and even with the same range with 9,917±1,165.

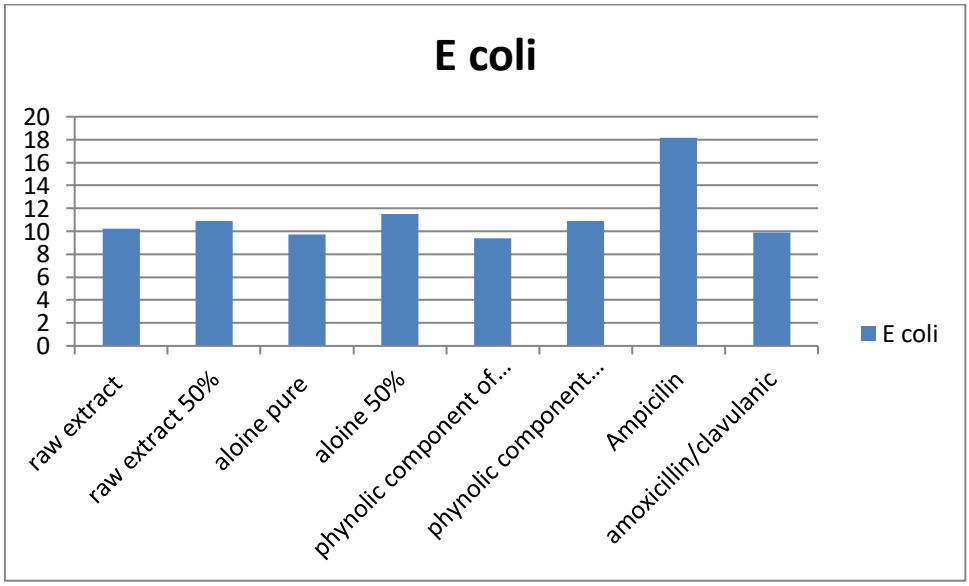


Figure 22: Average value diameter of *E coli* inhibition of by the different molecules studies (raw extract and diluted 50% ,aloin pure and diluted 50% phynolic component of latex pure and diluted 50% ,ampicilin and amoxicillin/clavulanic acid).

Discussion:

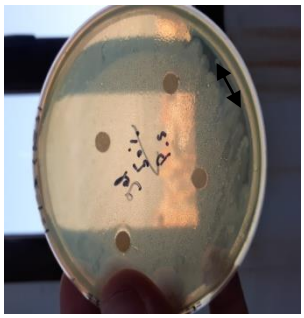
As we see that all the strains has been getting a sensibility against all the PPs molecules pure and diluted the same as what w saw on amoxicillin/clavulanic acid and with less compare to ampicilin that show a very sensitive.

And by that we can say *E coli* strain build or learned a future resistant if we keep using the same antibiotic on these strain of E coli otherwise we can use the PPs as bio antibiotic and safe for the human body.

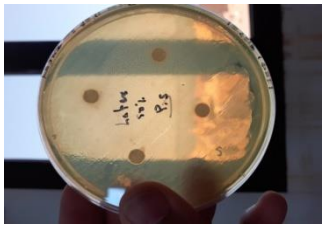
b. Comparative study between the effects of pure and diluted PPs studied on *P aeruginosa* and reference antibiotics:

DMSO

Raw extract 50%

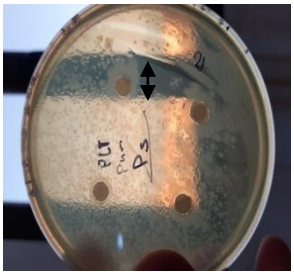


Aloine 50%

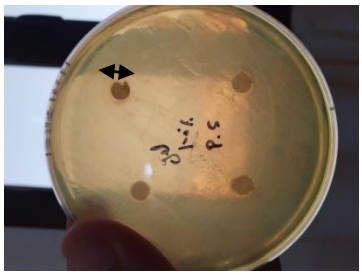


Phynolic component of latex 50%

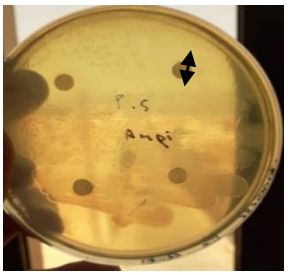
Phynolic component of latex pure



Plant total pure



The gel pure



Ampicilline

amoxicillin/clavulani

Figure 23: compare of the sensitivity of *P aeruginosa* to pure and 50% diluted PPs of aloe Vera and to reference antibiotics, marked by zones of inhibition.

Table 7 : Diameters of the inhibition zones in (mm). Marking by the sensitivity of P aeruginosa to pure and diluted PPs at 50% of aloe Vera and to reference antibiotics.

	Raw extract	Raw extrat 50%	Aloin pure	Aloine 50%	PLC pure	PLC 50%	Ampi	amoxicillin/clavulanic
P aeruginosa	7,25±3,793[a]	0,917±3,175[d]	3,5±4,503[c]	2,5±4,523[d]	7,5±4,964[a]	5,25±5,594[b]	10,75±4,393[a]	8,917±2,021[a]
Sensibility (±)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diametere.

(a,b,c ,d) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, results are expressed as means ± standard deviation

Sensitive (+), Very sensitive (++), Resistant (-), Extremely sensitive (+++)

Diameters of the inhibition zones marking by the sensitivity of *P aeruginosa* show a low values in all the molecules starting from 0,917±3,175 in raw extract 50% up to Phynolic component of latex pure 7,5±4,964 less the ampicilin and amoxicillin/clavulanic acid.

We see that raw extract and aloin 50%have the same significant value, raw extract and phynolic component of latex pure have the same significant value like the antibiotic of references ampicilin and amoxicillin/clavulanic acid.

For Aloin pure and Phynolic component latex 50% have different significant from the other and from each other.

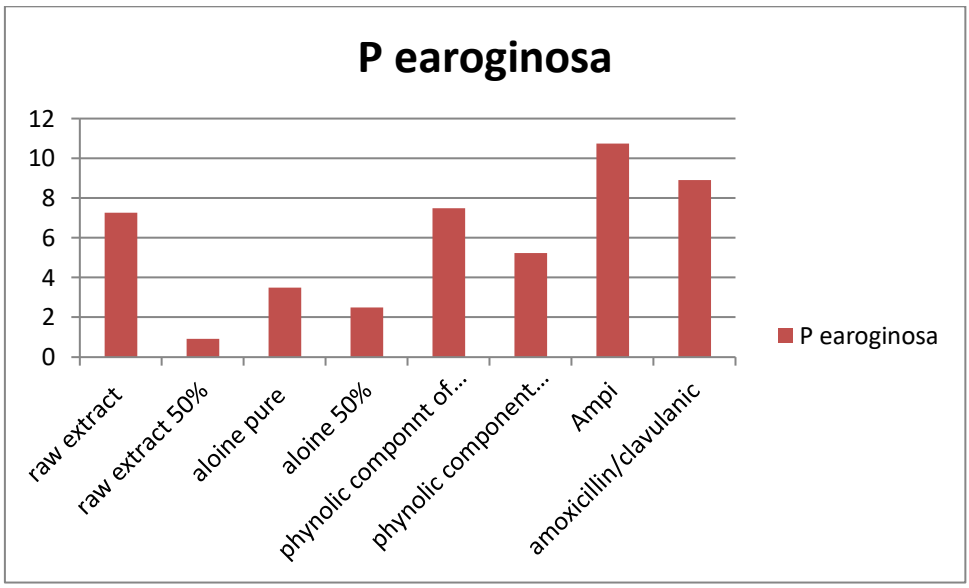
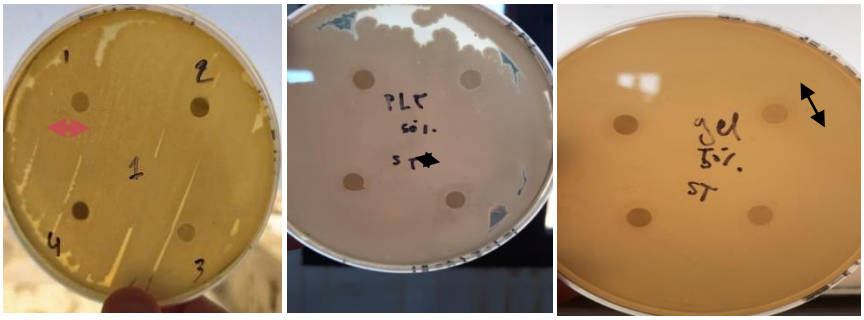


Figure 24: Average value diameter of *P aeruginosa* inhibition of by the diffrent molecules studies (raw extract and diluted 50% ,aloine pure and diluted 50% phynolic component pure and diluted 50% ,ampicilin and amoxicillin/clavulanic acid) .

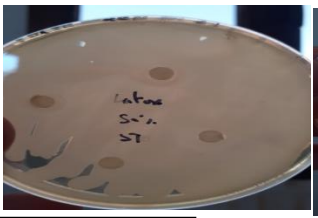
Discussion

We observe that the strain of *P aeruginosa* showed a resistant to all the molecule of PPs we studies like the antibiotic of reference amoxicillin/clavulanic acid get resisted by the strain of *P earoginosa* unlike the ampicilin show a sensitivity against but in his way to be resistant by if we study it on the generation of the same strain.

c. Comparative study between the effects of pure and diluted PPs studied on *S.aureus* and reference antibiotics:

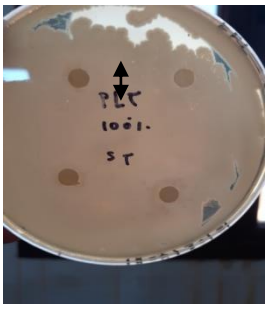


DMSO | Raw extract 50% | Aloine 50%



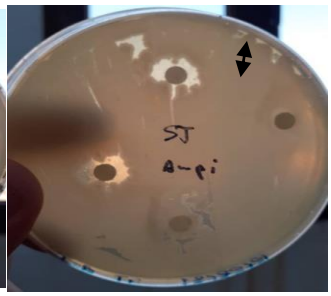
Phynolic component of latex 50%

Phynolic component of latex pure



Raw extract pure

Aloine pure



Ampicilline

amoxicillin/clavulanic

Figure 25: compare of the sensitivity of *S.aureus* to pure and 50% diluted PPs of aloe Vera and to reference antibiotics, marked by zones of inhibition.

Table 8: Diameters of the inhibition zones in (mm). Marking by the sensitivity of *S aureus* to pure and diluted PPs at 50% of aloe Vera and to reference antibiotics.

	Raw extract	Raw extract 50%	Aloine pure	Aloine 50%	PLC pure	PLC 50%	Ampicilin	amoxicillin/clavulanic
S-aureus	10,25 ±1,685^{c}	8,333±1,303^{d}	13,083 ±1,165^{b}	11±1,348^{c}	10,333± 1,67^{c}	10,083 ±3,801^{c}	17,5±2,714^{a}	9,183±3,363^{d}
Sensibility (±)	(+)	(-)	(+)	(+)	(+)	(+)	(++)	(+)

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diameters

(a,b,c ,d) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, results are expressed as means ± standard deviation

Sensitive (+), Very sensitive (++), Resistant (-), Extremely sensitive (+++)

Diameters of the inhibition zones marking by the sensitivity of S aureus as we see the diameters staring from maximum value 13,083±1,165 in the pure Aloin down to the lowes value 8,333±1,303 in the raw extract 50%, and as we see raw extract 50% and amoxicillin/clavulanic acid have the same significant value less then ampicilin 17,5±2,714 .

Raw extract pure ,aloin 50%, Phynolic component of latex pure and diluted 50% have the same significant value ,unlike aloin pure and diluted 50% have a different significant for the others and from each other.

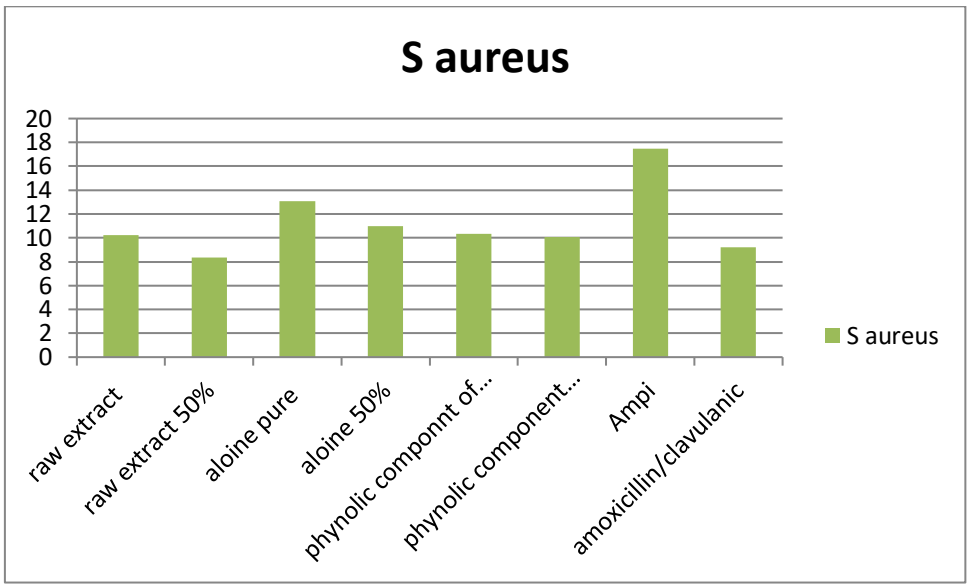


Figure 26: Average value diameter of *S auseus* by the different molecules studies Raw extract and diluted 50%, aloin pure and diluted 50% phynolic component pure and diluted 50%, ampicilin and amoxicillin/clavulanic acid).

Discussion:

We observed that raw extract diluted 50% and amoxicillin/clavulanic acid have the same significant but different sensitivity that's up to the strain starts to develop a resistant.

For the rest of the molicus showed a sensibility like the amoxicillin/clavulanic acid and less then ampocilin (very sensitive).

The stain of *S aureus* developed a resistant against the molecules we studies, comparing between the PPs we studies and antibiotic of reference its seems that we can replace the antibiotic with PPs extracted because it's give almost the same result and its bio and have no chemicals additives.

5.3. General discussion:

Table 9: Diameters of the zones of inhibition in (mm). Marked by the sensitivity of the different strains studied to pure aloe Vera PP's and diluted to 50%.

	Raw extract	Raw extract 50%	Aloin pure	Aloin 50%	PLC pure	PLC 50%	Ampi	amoxicillin/clavulanic
E. coli	10,25± 1,545^{b}	10,917± 2,109^{b}	9,75± 1,913^{b}	11,5± 2,355^{b}	9,417± 1,443^{b}	10,917± 1,24^{b}	18,167± 3,973^{a}	9,917± 1,165^{b}
P-aeroginosa	7,25± 3,793^{a}	0,917± 3,175^{d}	3,5± 4,503^{c}	2,5± 4,523^{d}	7,5± 4,964^{a}	5,25± 5,594^{b}	10,75± 4,393^{a}	8,917± 2,021^{a}
S-aureus	10,25±1, 685^{c}	8,333± 1,303^{d}	13,083±1, 165^{b}	11± 1,348^{c}	10,333±1 ,67^{c}	10,083± 3,801^{a}	17,5± 2,714^{a}	9,183± 3,363^{d}

The diameters of the discs (5mm) are included in the measurements of the inhibition zone diameters.

(a,b,c ,d) are homogeneous groups indicating a significant difference ($P<0.05$)

N = 3, results are expressed as means ± standard deviation.

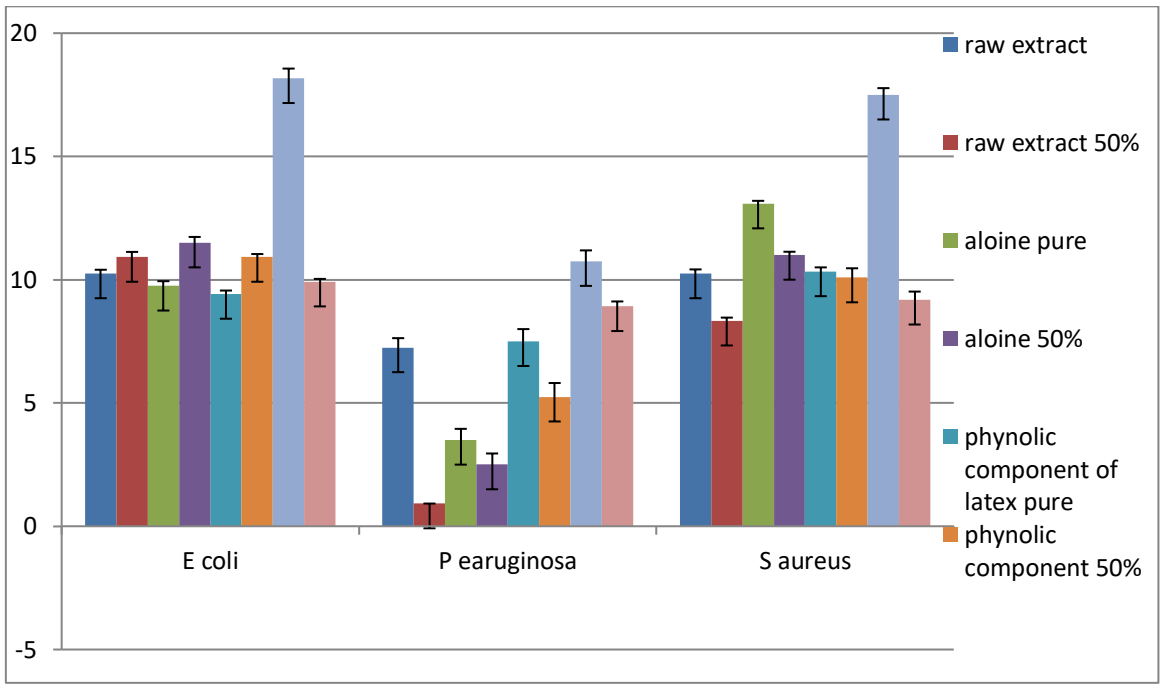


Figure 27: Average value diameter of *E coli* , *P aeruginosa* and *S aureus* inhibitions by the different molecules studies (raw extract and diluted 50% ,aloine pure and diluted 50% phynolic component pure and diluted 50% ,ampicilin & amoxicillin/clavulanic acid and their ecartypes).

The antibacterial effect of the phynolic component of the latex at 50% are very effective against the strain of P aeruginosa and the pure phynolic component of the latex with little less than the diluted 50%,that effect antibacterial very important even more then antibiotic of reference the ampicilin and amoxicillin/clavulanic acid and also the aloin pur and diluted give more effect then the antibiotic ampicilin and amoxicillin/clavulanic acid,raw extract pure and diluted 50% showed high effect more than amoxicillin/clavulanic acid and less then ampicilin.

Its seams that the ampicilin antibacterial effect significant high on the *E coli* strains its come as the most high value of inhibition compare to all other molecules that we studies.

E coli strain's against the antibacterial effect of raw extract pure and diluted 50% high effect then antibiotic amoxicillin/clavulanic acid but less then ampicilin and ampicilin.

The effect antibacterial of the phynolic component of the latex at 50% are very effective against the strain *S aureus* more effectively then the ampicilin and amoxicillin/clavulanic acid and for the rest of the PPs give less affectivity against the strain of *S aureus*.

According to the results of my trial, we can conclude that for most of the pathogenic strains, including the one targeted in my study, the polyphynols of Aloe Vera can be a good alternative to synthetic antibiotics. The latter can help us to fight against the problems of

antibiotic resistance, which diminish the credibility and reliability of antibiotic treatment, and also help us to avoid the side effects of the increased consumption of synthetic antibiotics

5.4. Conclusion and discussion:

Polyphenols are a family of organic molecules widely present in the plant kingdom. ... These compounds are the products of the secondary metabolism of plants. Polyphenols are becoming progressively more important, especially because of their beneficial effects on health, especially anti-bacterial effects. Its seems that the polyphynols of Aloe Vera rich with anthraquinone in the latex and aloin in the gel of Aloe Vera. The work carried out by my study allowed to put in evidence the antibacterial activities of PPs of Aloe Vera. So, the aloin pure and diluted 50% showed significant antibacterial activity against *S aureus* and the same on E coli but with is inferior to the *S aureus*, in the other hand it showed significant the lowest antibacterial effects compare to the other strains.

From the result it seems that PPs of aloe Vera work significant well against *P earoginosa* and *E coli* in other hand Polyphenols of aloe Vera showed disability against the strains of *P earoginosa* and it get resisted. The results of the tests of the application of PPs (raw extract, aloin,phynolic compounds of latex) on our target strains *E. coli, Ps. Aeruginosa and S. aureus*.

Have proved to be very interesting by its antibacterial strenght. From these results, we can conclude that the PPs of Aloe Vera seems to be more suitable as natural antibacterial agent in the prevention and cure of bacterial infections. New perspectives can be envisaged by a further study of the antibacterial activity not only on PPs used alone or their major components, but also in mixture, thus allowing a possible synergy. It would be interesting to continue this work, especially on other pathogenic bacteria, in order to confirm the effectiveness or not of Aloe Vera PP's.

The use of polyphynols as an alternative to synthetic antibiotics, of which the majority of pathogenic bacteria have developed a resistance, which has reduced their effectiveness in the fight against the spread of bacterial infestations.So the use of polyphenols could be considered in the pharmaceutical and medical field.

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